DISSERTATION ON
IMMUNOHISTOCHEMICAL ANALYSIS OF MASPIN EXPRESSION IN CHRONIC NON NEOPLASTIC AND NEOPLASTIC COLORECTAL DISEASES

A STUDY OF 70 CASES

Dissertation submitted to

TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY
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Under the guidance of

Dr. NALLI .R. SUMITRA DEVI, M.D.,
Professor,
Department of Pathology
Govt. Stanley Medical College
Chennai

THE TAMILNADU Dr.M.G.R.MEDICAL UNIVERSITY
CHENNAI – TAMILNADU

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CERTIFICATE

This is to certify that this dissertation titled "Immunohistochemical Analysis Of Maspin Expression In Chronic Non Neoplastic And Neoplastic Colorectal Diseases"is the original and bonafide work done by Dr.Deepa.R under the guidance of Dr.Nalli.R.Sumitra Devi, M.D., Professor, Department of Pathology at the Government Stanley Medical College & Hospital, Chennai-600 001, during the tenure of her course in M.D.Pathology from May 2012 to April 2015 held under the regulation of the Tamilnadu Dr.M.G.R. Medical University, Guindy, Chennai-600032

PROF.S.MARY LILLY,M.D
Professor and Head
Department of Pathology
Government Stanley
Medical College
Chennai-600 001

PROF.AL.MEENAKSHI SUNDARAM.M.D.D.A,
DEAN
Government Stanley Medical College
Chennai-600 001

Place:Chennai
Date: .09.2014

Place:Chennai
Date: .09.2014

CERTIFICATE BY THE GUIDE

This is to certify that this dissertation titled DISSERTATION ON "Immunohistochemical Analysis Of Maspin Expression In Chronic Non Neoplastic And Neoplastic Colorectal Diseases - A STUDY OF 70 CASES" is the original and bonafide work done by Dr.Deepa.R under my guidance and supervision at the Government Stanley Medical College & Hospital, Chennai-600 001, during the tenure of her course in M.D.Pathology from May 2012-April 2015 held under the regulation of the Tamilnadu Dr.M.G.R. Medical University, Guindy, Chennai-600032

PROF.NALLI R. SUMITRA DEVI
Professor
Department of Pathology
Government Stanley Medical College
Chennai - 600 001

Place:Chennai
Date: .09.2014

DECLARATION BY THE CANDIDATE

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Place:Chennai Signature by the candidate

Date: .09.2014 Dr.Deepa.R

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CONTENTS

ABBREVATIONS

DNA	-	Deoxyribonucleic Acid
HOX	-	Homeobox
SHH	-	Sonic Hedgehog
GIT	-	Gastro intestinal tract
IRGM	-	Immunity related GTPase M
ATG16L1	-	Autophagy related 16 like
NOD2	-	Nucleotide-binding oligomerization domain-containing protein 2
FAP	-	Familial Adenomatous Polyposis
APC	-	Adenomatous Polyposis Coli
KRAS	-	Kirsten rat sarcoma
HLA	-	Human Leucocyte Antigen
TGF Beta	-	Transforming growth factor Beta
AJCC	-	American Joint Committee on Cancer
IBD	-	Inflammatory Bowel Disease

5FU	-	5 Fluorouracil
IHC	-	Immunohistochemistry
HRP	-	Horse Radish Peroxidase
EDTA	-	Ethylene diamine tetra acetic acid
TBS	-	Tris Buffered Saline
DAB	-	3,3' - Diaminobenzidine
DCC	-	Deleted in colon cancer
MSI	-	Microsatellite instability
CIMP	-	CpG Island methylator Pathway
CIN	-	Chromosomal instability

ABSTRACT

BACKGROUND

Maspin is found to be a member of serine protease inhibitor/ non inhibitor superfamily like plasminogen activator inhibitors 1 and 2 and alpha-1 antitrypsin.[4]The gene for maspin is located on chromosome 18q21.3.It has been shown to be involved in processes that are important to both tumor growth and metastasis such as apoptosis, cell invasion and angiogenesis.

This study is aimed to analyse maspin expression in chronic non neoplastic and neoplastic colorectal diseases.

AIMS AND OBJECTIVES

1. To study tumor progression in colorectal adenocarcinomas.
2. To evaluate maspin expression and its correlation with clinicopathologic parameters in chronic non neoplastic and neoplastic colorectal diseases.
3. To evaluate the prognostic value of immunohistochemical expression of maspin in chronic non neoplastic and neoplastic colorectal diseases.

MATERIALS AND METHODS

A total of 70 cases including 10 controls (normal colonic mucosa) of non neoplastic and neoplastic colorectal diseases were used for immunohistochemical analysis of maspin expression .The cases included are Non specific colitis, Ulcerative colitis, colorectal adenomas and adenocarcinomas received in the Department of Stanley Medical College from

the Department of Surgery in the year 2012-2013. The immunoreactivity to maspin was identified by staining of the cytoplasm. 100 cells were randomly selected and counted from 5 representative fields

RESULTS

In our study maspin expression was found to be increased in males,increasing depth of invasion , presence of lymphatic and vascular invasion and poor survival,thus showing increased expression with increase in aggressiveness of colorectal cancers.Also increased expression was found in carcinoma compared to normal colonic mucosa, non specific colitis and ulcerative colitis. No significance was found to be associated with increasing tumor size or differentiation of the tumor or presence of liver metastasis. Our study is comparable with other parallel studies where expression of maspin was studied in colorectal carcinoma.

CONCLUSION

The expression of maspin with various clinicopathological parameters was analysed and its importance as a prognostic factor was assessed.To conclude in our study maspin was found to be associated with increasing aggressiveness of colorectal cancers.

KEY WORDS : Ulcerative colitis , Adenoma , Colorectal carcinoma, immunohistochemistry , Maspin.

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KEY WORDS : Ulcerative colitis , Adenoma , Colorectal carcinoma, immunohistochemistry , Maspin.

INTRODUCTION

Colorectal carcinoma, Adenoma and Inflammatory bowel diseases are common in the Western industrialized nations.[1,2,3] However the prevalence is getting higher in regions such as Asia, Africa and South America.

The incidence of carcinoma is equal in males and females.[2] The causes for development of carcinoma are varied and include both genetic and environmental factors. Epithelial polyps and inflammatory bowel disease have a definite predisposition to colorectal carcinoma.[2] This transformation includes mutational activation of oncogenes and inactivation of tumor suppressor genes.

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In certain cases maspin was found to be paradoxically overexpressed in active Inflammatory bowel disease, colitis associated

dysplasia and it was correlated with high Duke's classification ,depth of invasion and high grade tumor budding in colorectal carcinomas, thereby correlating with its aggressiveness.

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REVIEW OF LITERATURE

Embryology

Divisions of the Gut Tube

Primitive gut is formed by the cephalocaudal and lateral folding of the embryoleading to incorporation of a portion of the endoderm-lined yolksac cavity into the embryo .The other portions of theendoderm-lined cavity namely the **yolk sac** and the **allantois,**remain outside the embryo . The middle part, the **midgut,** remains attached to the yolk sac by means of the **vitelline duct** whereas in the cephalic and caudal parts of the embryo, theprimitive gut forms a blind-ending tube, the **foregut** and**hindgut,** respectively**.**Development of the primitive gut and its derivativesis discussed in four sections: The **pharyngeal gut,**whichextends from thebuccopharyngeal membrane tothe tracheobronchial diverticulum.The **foregut that**lies caudal to the pharyngealtube and extends to the liver outgrowth. The **midgut that** begins caudal to the liver bud and extends to the junction of the righttwo-thirds and left third of the transverse colon in the adult. The**hindgut which** extends from the left third of the transverse colon to the cloacalmembrane.

It is the Mesoderm which dictates the type of structure that will form through a ***HOX* code.** Differentiation of the various regions of gut and its derivatives dependson a reciprocal interaction between the endoderm of guttube and the surrounding splanchnic mesoderm. **This HOX code is similar** to the one thatestablishes the anterior and the posterior body axis. Induction of the *HOX* code is a result of ***sonic hedgehog (SHH)that is***expressed throughout the gutendoderm. Thus, in the region of the mid- and hindgut, expression of *SHH* ingut endoderm leads to a nested expression of the *HOX* code in the mesoderm.After themesoderm gets specified by this code, it instructs theendoderm to form the various components of the mid- and hindgut regions,which includes the small intestine, cecum, colon, and cloaca.

Portions of the gut tube and its derivatives are suspended from dorsal andventral body wall by **mesenteries**.Such organs are called **intraperitoneal,**whereas retroperitoneal organs are those that lie against the posterior body wall and are coveredby peritoneum on their anterior surface only**. Peritoneal ligaments** are double layers of peritoneumthat pass from one organ to another or from an organ to thebody wall.Initially the foregut, midgut, and hindgut are in broad contact with the mesenchyme of posterior abdominal wall .By the fifth week, the

connecting tissue bridge gets narrowed and the caudal part of the foregut, the midgut and a major part of the hindgut are suspended from the abdominal wall by the **dorsal mesentery** which extends from lower end of the esophagus to the cloacal region of the hindgut. In the region of the stomach it forms the **greater omentum;**in the region of the duodenum it forms the dorsal **mesoduodenum;** andin the region of the colon it forms the **dorsal mesocolon.** Dorsal mesentery of the jejunal and ileal loops forms the **mesentery proper**[5]**.** Mesenteries and ligaments provide pathways for vessels, nerves,and lymphatics to and from abdominal viscera.

Anatomy

The adult human esophagus measures 40 cms from incisor teeth to the gastro esophageal junction. The upper esophageal sphincter is at the level of cricopharyngeal muscle. The lower esophageal sphincter is 2 to 3 cms above the gastroesophageal junction. The stomach is a sacular organ with potential capacity of 3000 ml. It begins at the gastro esophageal junction and ends at the pylorus where the muscles get thickened to form the pyloric sphincter.The small intestine is 6 metres in length. It begins with the duodenum which is retroperitoneal and extends for about 25 cms and then continues as the jejunum which enters the peritoneal cavity till the ileoceacal valve where it transforms into colon and becomes retroperitonal again.

The colon

The colon is composed of Ceacum, ascending colon, transverse colon and descending colon. The ceacum and ascending colon are covered on their ventral surfaces by peritoneum but posteriorly they are directly fixed to the posterior abdominal wall. The transverse colon extends from the hepatic flexure to the splenic flexure and gets suspended by the lesser omentum. The descending colon is adherent to

the posterior abdominal wall. The sigmoid colon is the only portion of colon which is suspended by the mesentery and begins as the pelvic brim. It then continues as the rectum which ends with the anal canal.[6]

Physiology

The main function of GIT is to help the body to absorb nutrients, water, vitamins and minerals. So predominantly the entire work of the Gastro intestinal system is divided into digestive work and absorption of products of digestion.The digestive process is accomplished by mechanical and chemical means. The mechanical means includes the acts of chewing ,swallowing and movements of GIT. The chemical means are achieved by saliva , gastric juice , bile juice and intestinal juice .

The large intestine predominantly involves mixing movements due to the haustrations for exposure of the feacal material to the surface of the large intestine.The propulsive movements help in transporting the fecal material further down the colon . Gastrocolic and duodenocolic reflexes helped in distension of stomach and duodenum and further initiation of mass movements.

The distention of rectum is brought about by defecation reflex which generates a pressure of 50 – 60 mm Hg . This reflex is caused by activation of parasympathetic nerve fibres which cause relaxation of internal anal sphincter. Finally,Voluntary relaxation of the external anal

sphincter associated with increased pressure of the abdominal wall muscles cause the act of defecation.[7]

Histology

The histology of the colon is such that it can perform its principal function of absorption of salt and water from the feces and propulsion of solid feces to the rectum before defecation.The layers of large intestine includes the four layers – Mucosa which is further subdivided into epithelium, lamina propria and muscularis mucosae, Sub mucosa, Muscularis propria and the serosa.The muscularis layer is divided into inner circular and outer longitudinal bands. The longitudinal layer is further divided into three longitudinal bands called the teania coli. Teania coli are not found in the rectum.

The mucosa forms longitudinal folds of columns of morgagni immediately above the anal valves.The mucosa consistes of two types of cells (i) Absorptive cells and (ii) the mucus secreting goblet cells. They are arranged in straight tubular glands or crypts which extend upto the muscularis mucosae. The alcian blue stain can be used to differentiate between the two. The goblet cells stain greenish blue whereas the absorptive cells do not take up any color.

The rectal mucosa has more number of goblet cells compared to the rest of the intestine.At the recto anal junction the branched

circumanal glands open at the distal end of columns of morgagni into small pits. The anal canal forms last 2-3 cms of the Gastrointestinal tract and here the stratified squamous epithelium transforms to skin containing sebaceous and large apocrine sweat glands.[8]

Pathology:

Inflammatory bowel diseases :

With the increasing use of flexible sigmoidoscopy and colonoscopy pathologists have an increasingly important role in the diagnosis of colitis. Before we go into the pathology of inflammatory bowel disease, following is a brief introduction to the normal findings in intestinal mucosa.

The luminal surface is straight and the Colonic tubules are found to be tightly packed, parallel and are nonbranching. Goblet cells are numerous. These glands are closely associated with the muscularis mucosae. The appearance can be equated to test tubes in a rack. The lamina propria contains a mixed inflammatory cell infiltrate, including plasma cells, lymphocytes, eosinophils, and macrophages which are proportionately less in number.

Rarely an intraepithelial lymphocyte can be present. The muscularis mucosae is thin. The submucosa is generally devoid of inflammation. Scattered intramucosal lymphoid follicles can be encountered, mainly in younger individuals. The architecture may be distorted in areas of lymphoid follicles, the muscularis mucosae may be incomplete and some of the lymphoid follicles may spill over into the

submucosa. Overlying the lymphoid aggregates are flattened surface cells called M cells. In this M-cell region, the epithelium normally contains more number of mononuclear inflammatory cells, and the amount of intraepithelial mucin is decreased .Paneth cells are considered a normal finding only in the cecum and the proximal ascending colon.[9]

Inflammatory bowel diseases includes those disorders which cause inappropriate mucosal immune response againstnormal commensal bacteria. This is supported by the theory of hygiene hypothesis which states that better storage of food and decreased contamination of food leads to overwhelming immune response to normal commensals in susceptible individuals because of inadequate regulatory processes.[10] Two conditionsare predominantly included in this– Crohn's disease and Ulcerative colitis. Our major concern is Ulcerative colitis as it has more potential to transform into malignancy than Crohn's disease.Hence cases of Ulcerative colitis have been included in the study. The following is a brief outlook on the pathogenesis, gross, microscopic picture and the course of the disease.

Pathogenesis

Genetic factors : Polymorphisms in NOD2, IRGM (immunity related GTPase M) and ATG16L1 (Autophagy related 16 like) are found to be responsible for increasing inflammatory activity and abnormalities in the epithelial barrier mechanisms. These changes are more related to Crohn's than Ulcerative colitis.

Mucosal immune Responses : T_h1, T_h17 are involved in the immune dysregulation in Crohn's as well as Ulcerative colitis. Hence mutations in IL-23 seems to confer some protection in both these conditions. Also T_h2 seems to be involved in Ulcerative colitis due to increasing IL-13 activity associated with it.

Epithelial defects : Mutation of organic cation transporter and mutation of extracellular matrix protein 1 which inhibits matrix metalloproteinases-9 are associated with Ulcerative colitis.

Microbiota: Antibodies against bacterial protein flagellin are associated with colitis manifestations.[11]

Ulcerative colitis : More commonly is associated with malignant potential that Crohn's disease. Ulcerative colitis occurs with equal frequency in both sexes. It appears most often in patients between 20 and 30 years of age, with a second peak between 70 and 80 years. Ulcerative colitis is characteristically a left-sided disease, which usually begins in the rectosigmoid area.

Grossly the mucosa is red and granular and has broad based ulcers. The ulcers are longitudinal and not serpentine like in Crohn's disease. Pseudopolyps and mucosal regenerative bridges are present. Mucosal atrophy takes place in chronic conditions and damage to the muscularis propria disturbs the neuromuscular function and leads to toxic megacolon.[12]

Microscopic finding shows dense lymphoplasmacytic infiltrate in mucosa and submucosa. Architecturally distorted glands containing intraepithelial and luminal lymphocytes indicating cryptitis and crypt abcess.[13]The density of neutrophils is similar throughout the mucosa and submucosa leading to equalized mucin depletion and occasional crypt rupture can lead to accumulation of histiocytes and giant cells can result in mucin granulomas which are different from those seen in Crohn's disease.[14]In fulminant diseases the inflammation may extend

beyond muscularis mucosa. Crypt architectural distortion,basal plasmacytosis,paneth cell metaplasia and basally located lymphoid aggregates are considered as markers of previous significant crypt injury.Hence these are considered as markersof chronic colitis.[15]

Patients with Chronic Ulcerative colitis are at an increased risk for dysplasia and then transformation to adenocarcinoma.[16,17,18] The biopsy specimens are classified into the following three groups depending upon the changes present :

1.Positive

2. Negative

3. Indefinite for dysplasia

Positive dysplasia is further classified into high grade and low grade dysplasia depending upon extent of dysplastic changes. Low grade dysplasia is limited to the lower half of the epithelium whereas high grade dysplasia extends to the luminal side of the epithelium with increased stratification, cytologic atypia and loss of nuclear polarity.

Indefinite for dysplasia is further divided into probably dysplastic, probably inflammatory and unknown[16]. Hence the need for endoscopic surveillance in long standing cases of Ulcerative colitis.This

dysplasia can be present in a flat mucosa or mucosa with villous configuration or mucosa with nodular growth[16].

The following is the standard surveillance protocol for colitis patients which has been widely recommended[17,19,20].

Table 1

Mucosal Ulcerative Colitis and Dysplasia: Management Based on Biopsy taken for surveillance

Biopsy Interpretation	*Recommendation*
Negative for dysplasia	Regular follow-up to be continued
Indefinite for dysplasia	Follow-up for short term.
Positive; low-grade dysplasia	Follow-up for short term; some recommend colectomy; others consider colectomy if associated with suspicious gross lesion[a]
Positive; high-grade dysplasia	colectomy[a] to be considered

Adenomas

Table 2

Classification of Serrated Colonic Polyps

I. **Nondysplastic Serrated Polyps**

A. Normal architecture, normal proliferation

1. Microvesicular hyperplastic polyp

2. Goblet cell hyperplastic polyp

3. Mucin-poor hyperplastic polyp

B. Abnormal architecture, abnormal proliferation

1. Sessile serrated polyp

II. **Dysplastic Serrated Polyps**

A. Sessile serrated polyp with dysplasia (mixed polyp,advanced sessile serrated polyp)

B. Serrated adenoma (traditional)

C. Conventional adenoma with serrated architecture

III. **Unclassifiable Serrated Polyp** (either with or withoutdysplasia)

This study includes adenomatous polyps as these polyps are characterized by dysplasia and hence have more chances of progression to adenocarcinoma.

Adenomas are mainly classified into conventional, serrated or flat.Conventional adenomas are further classified into tubular, tubulovillous and villous adenomas.When multiple adenomas are present it may indicate a genetic syndrome (FAP, attenuated FAP,MYH-associated polyposis syndrome). Prevalence increases after the age of 40 years.[21]The presence of one adenoma is associated with 40–50% increased risk of additional adenomas.The risk for new adenomas is 30-60% after polypectomy for initial adenoma.[21]

Clinically most patients have rectal bleeding.Hence Large polyps can cause iron deficiency anemia[22].

Grossly adenomas are exophytic mucosal protrusions.Large adenomas can be hemorrhagic and adenomas larger than 2 cms are more likely for malignant transformation than smaller adenomas.[23]

Microscopic description: The villous type of adenoma is characterized by more than 75% villous architecture and the tubular type by less than 25% villous architecture. Hence the tubulovillous type has between 25–75% villous architecture.Adenomas are typically characterized by the presence of dysplasia.It can be high grade or low grade. High grade dysplasia have significant pleomorphism, increase in

mitotic activity,numerous atypical mitoses and increased loss of polarity. Architectural changes such as back-to-back gland configuration and cribriforming can also be noted. With progression of neoplasia,glandsbecome more irregular and complex and lose their orderly configuration. In addition, neoplastic nuclei become more “open” in appearance and may contain prominent nucleoli.Intramucosal adenocarcinoma show invasion into the lamina propria which will not be present in high grade dysplasia[24].

True adenomas which are characterized by serrated adenomas are more commonly left sided .The lining cells show high nuclear to cytoplasmic ratio with abundant mucin, high nuclear pleomorphism and increased mitotic activity.[25]

The evaluation of an endoscopically removed malignant polyp is a stepwise process that involves

(a) adequate fixation,

(b) sectioning,

(c) knowledge of the type of removal,

(d) examination of slides, and

(e) pathologist-clinician interaction.

When examining the slides, one should evaluate and report on the following:

(a) status of resection margin

(b) grade of cancer, and

(c) presence or absence of lymphatic or venous invasion.

As the treatment varies depending upon the presence or absence of the above factors. Presence of lymphatic invasion will lead to conversion of polypectomy to extended colectomy to prevent further spread of the disease.[26] Biologically, adenomatous growth is thought toprogress sequentially, through a continuum: lowgrade dysplasia, high-grade dysplasia, carcinoma insitu, intramucosal carcinoma, and invasive carcinoma.[27]

Some of the syndromes associated with adenomatous polyps include Familial adenomatous polyposis, Turcot's syndrome and Gardner's syndrome.

Adenocarcinoma

Adenocarcinomas of the colon and rectum are more common in the industrialized countries than in other developing countries. Asia stands as a low risk region when it comes to this malignancy. Colorectal carcinomas occur with equal frequency in both men and women and the mean age of occurrence is found to be 62 yrs.[28] Although in the developing countries it can be found from 50 yrs of age.[29]Carcinomas occurring in distal colon and rectum are found to be more aggressive compared to those occurring in other regions.[28]

The risk factors for occurrence of colorectal carcinomas are found to be the following-

Table 3[30]

The risk factors for occurrence of colorectal carcinomas

	Relative risk
Family history	**1.8**
Physical inactivity	**1.7**
Inflammatory bowel disease	**1.5**
Obesity	**1.5**
Red meat	**1.5**
Smoking	**1.5**
Alcohol	**1.4**
High vegetable consumption	**0.7**

Other causes include oral contraceptive use, estrogen replacement and pelvic irradiation. Consumption of Non steroidal anti-inflammatory agents seems to have a protective effect by interfering with prostaglandin homeostasis in colorectal cancers. Certain polymorphisms in enzymes can have protective or deleterious effects on colorectal carcinomas.

The genetic syndromes that have association with colorectal carcinomas include the following :

1. Hereditary non polyposis colorectal cencer syndrome with mutations in the DNA repair genes including MSH2,MSH6, MLH1.

2. Familial Adenomatous polyposis syndrome and Attenuated familial adenomatous polyposis syndrome that includes mutations in the APC gene.

3. Torre-Muir syndrome that is associated with several keratoacanthomas and sebaceous tumors[31]

4. Certain other syndromes includes Juvenile polyposis syndrome, Cowden syndrome, Puetz jeghers syndrome with an attributable risk of much less that 0.5 %.[32]

Pathogenesis

The occurrence of colorectal carcinomas is associated with certain genetic abnormalities like genomic instability that leads to chromosomal alterations and microsatellite instability that is caused by mismatch DNA repair.

The progression of adenoma to carcinoma is characterized by the following changes :

Initially the normal epithelium undergoes a 5q mutation that leads to formation of hyperproliferative epithelium then it undergoes DNA hypomethylation to progress to a low grade adenoma then it undergoes mutation in 12p or KRAS to progress to an intermediate adenoma. Mutation or loss of 18q leads to development of a high grade adenoma. Ultimately mutation in 17p and p53 leads to carcinoma and later on other accumulated mutations leads to metastasis.[33]

Clinical Presentation : The most common form of presentation is anemia. Right sided tumors bleed easily and thus present with anemia compared to left sided tumors that cause changes in bowel habits and can lead to melena.[34]Other than that patient can develop fever, malaise, weight loss and abdominal pain.

Gross: All tumors which are within 15 cms of the anal verge and in the non peritonalised portion of the left colon are considered to be rectal in origin. Most of the tumors are rectosigmoid in origin. The varied gross appearances can be:

1. Bulky polypoid and exophytic tumors
2. Infiltrating and ulcerating tumors
3. Constricting tumors that produce proximal dilatation of the colon
4. Diffuse tumors like linitis plastica

Microscopic features : This neoplasm is characterized by malignant glands invading the submucosa from the muscularis mucosae. The glands are lined by malignant columnar epithelial cells.

Adenocarcinoma is further graded :

Grade 1 : Glands forming more than 95 % of the tumor

Grade 2 : Glands forming 50 – 95 % of the tumor

Grade 3 : Glands forming 5-50 % of the tumor

Grade 4 – Glands forming less than 5 % of the tumor

By default Signet ring cell carcinoma and Mucinous carcinoma are included in Grade 3 or Poorly differentiated

category.Medullary carcinoma, Undifferentiated carcinoma and Carcinosarcoma, Adenosquamous carcinoma are other histological types. Other rare histopathological variantsof colorectal carcinoma include pleomorphic (giant cell) type, choriocarcinoma,pigmented, clear cell, stem cell, andPaneth cell-rich (crypt cell carcinoma).Mixtures of these variants can be seen.[35]

As this paper deals with the prognostic significance of maspin in colorectal diseases, a brief review about some of the other factors that play a major role in determining the prognosis-

Factors associated with poor prognosis include :

1. **Age** : Tumors in very young and in very old age groups
2. **Sex** : Tumors in males
3. **CEA levels** : > 5 ng / dl are associated with poor prognosis
4. **Tumor location** : This concept is controversial but one large study concluded that tumors in the rectum and sigmoid are associated with worse prognosis compared to tumors in right colon.[36] Another study stated that tumors in the left colon are associated with a higher rate of recurrence compared to other tumors.[37]

5. **Obstruction and perforation :** Are associated with poor prognosis
6. **Tumor local extent** : Tumor extending beyond bowel wall and to the adjacent lymph nodes have a poor prognosis
7. **Tumor edge** : Advanced tumors with a non polypoid edge are said to have a poor prognosis
8. **Tumor Budding** : Tumor cells > 5 at the invasive tumor front are said to have poor prognosis
9. **Vascular invasion**,**Perineural spread**, **Pericolonic tumor deposits** are said to have poor prognosis
10. **Tumor thickness** : Thickness of the tumor at the central depressed area is said to predict the presence of metastasis and lymph node involvement
11. **Tumor angiogenesis**
12. **Positive surgical margins** and in rectal adenocarcinomas presence of tumor less than 2mm from the circumferential margin is associated with poor prognosis.
13. **Microscopic tumor type** : Signet ring cell carcinoma, Mucinous carcinoma and Anaplastic carcinomas are associated with poor prognosis.

14. **Microacinar morphology** is associated with poor prognosis

15. **Mucin related antigens** : Sialyl Tn, Sialyl Lewis are associated with a more aggressive course.

16. **Fascin** is associated with poor prognosis

17. **pRB,P16^{INK4},KRAS, DNA aneuploidy, Claudin 1** expression and allelic loss of chromosome 18q are associated with poor prognosis.

18. **Lymph node involvement and high microscopic grade** and stage are associated with poor prognosis.

Some of these factors would be assessed in the present study along with maspin expression.

Factors responsible for good prognosis include the following :

1. **Sex** : Females have a better prognosis

2. **Margins and inflammatory infiltrate** : presence of pushing margins and a predominant inflammatory infiltrate at the periphery of the tumor is associated with a better prognosis.

3. **HLA-DR expressions, Bcl2 Expression and TGF beta 1 mutations** are all associated with better prognosis.

4. **Pattern of lymph node reaction** : Those patients in whom the lymph nodes show a predominant cell mediated immune reaction characterized by prominent immunoblasts and sinus histiocytosis have a better survival rate than others.[38]

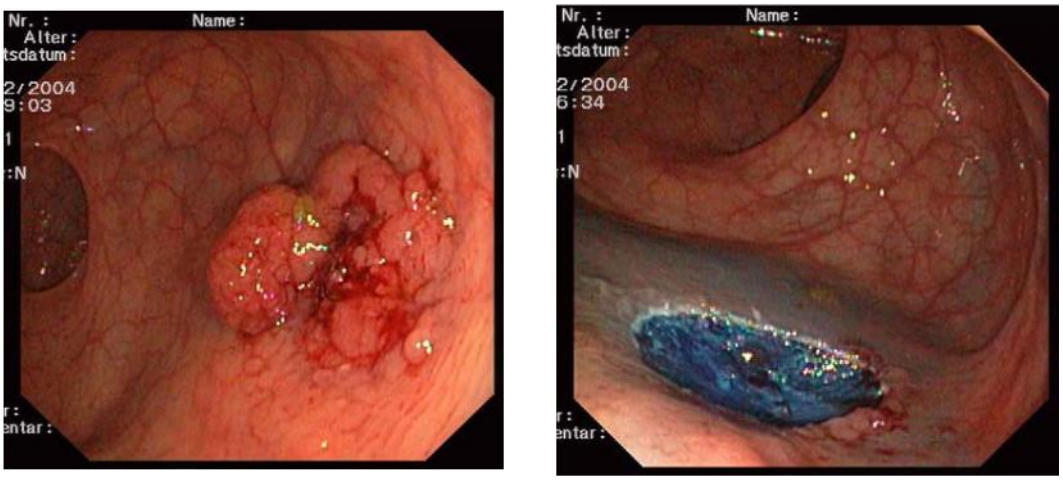
ADENOMA CARCINOMA SEQUENCE

General points in favour of the transformation :

Clinical Evidence of the sequence

- Adenomas and Carcinomas are known to exist in Parallel.
- Adenomas are found in patients who are 7 to 8 years younger than the carcinoma patients.
- Resected Carcinomas are found contiguous with, Benign adenomatous tissue.
- Adenomas precede Carinomas in pts with FAP and HNPCC and are found to have the same general histology as found in isolated sporadic carcinomas.

Figure 1 : Picture in the left showing benign polyps and Picture in the right shows the same polyp which has progressed to cancer as it was not resected.



The events associated with transformation of adenoma to carcinoma are distinct molecular alterations associated with Well characterized series of histopathologic events.

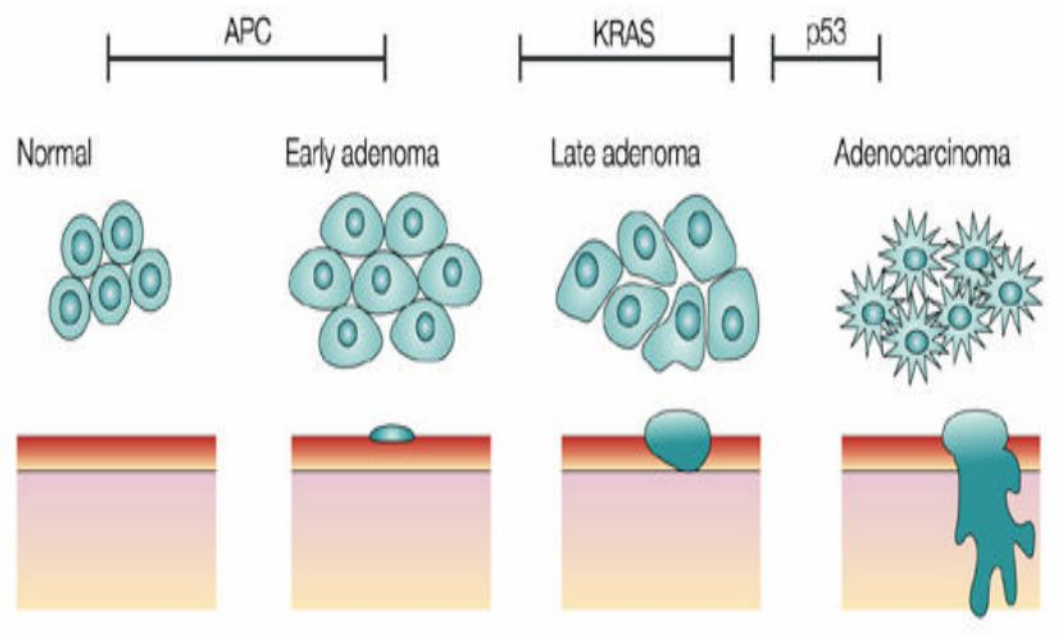
- Sporadic colorectal carcinoma (CRCs) can be subclassified into three types based upon the following genetic abnormalites:

(A) Microsatellite instability / MSI

(B) Chromosomal instability / CIN

(C) An additional group with CpG island methylator phenotype / CIMP

Figure 2 : An overview of the mutations that occur



(A) Chromosomal instability pathway

- Carcinomas arise from the accumulation of activation (by mutation) and inactivation of oncogenes and tumor-suppressor genes respectively that initially cause adenomatous polyps;then some acquire additional mutations and become malignant. A total of 4-10 mutations are required to produce malignancy.
- **APC** gene mutation is the earliest event .(germline mutations of APC are responsible for familial adenomatous polyposis (FAP) syndrome, while somatic mutations of APC occur in 50% of sporadic adenomas and 80% of sporadic colon cancer)
- **DCC** (Deleted in colon cancer) gene loss occurs later in carcinogenesis . It is frequently deleted in carcinoma (73%) and a lower percentage is associated with high grade adenoma (47%).
- **K-ras** mutations (10% of adenomas, 50% of adenomas with severe dysplasia, 35-45% of carcinomas) occur in larger polyps.
- KRAS is the downstream effector of EGFR, and hence in colorectal cancer, the mutational status of KRAS has become an important predictive marker for the effectiveness of chemotherepeutic drugs for metastatic colorectal cancers
- **p53 gene mutation** is found in 50% of adenomas with high grade dysplasia and 70% of sporadic colon cancers

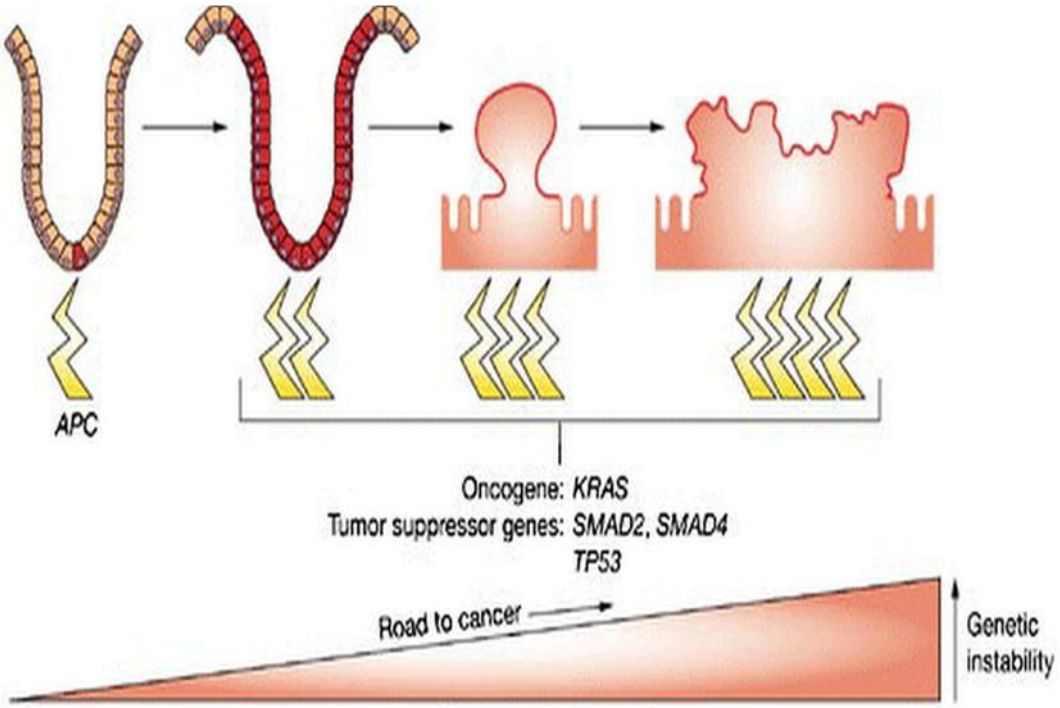
- Loss of the wild type of p53 activity leads tofailure of response to radio- and chemotherapy.
- **DPC4/DCC/SMAD4 mutation** (18q21, reduced expression in 70% of carcinomas) which occurs later in the sequence indicates advancing malignant phenotype.
- Loss or low level of SMAD4 expression in colorectal carcinomas is associated with poor prognosis.
- **EGFR**, a receptor tyrosine kinase, overexpression is seen in up to 80% of colorectal cancers and is associated with poor prognosis.
- Screening Colonoscopy to remove adenomas reduces the incidence of colorectal cancer.
- Polyposis syndrome patients and Villous adenomas have an increased risk for carcinoma (nearly 100% for familial polyposis and Gardner's syndrome).
- ***Polyps which have no malignant risk are the following:***

solitary hyperplastic polyps,

juvenile polyps and

Peutz-Jegher polyps

Figure 3 : Sequence of events after the earliest APC mutation



(B) Microsatellite instability pathway

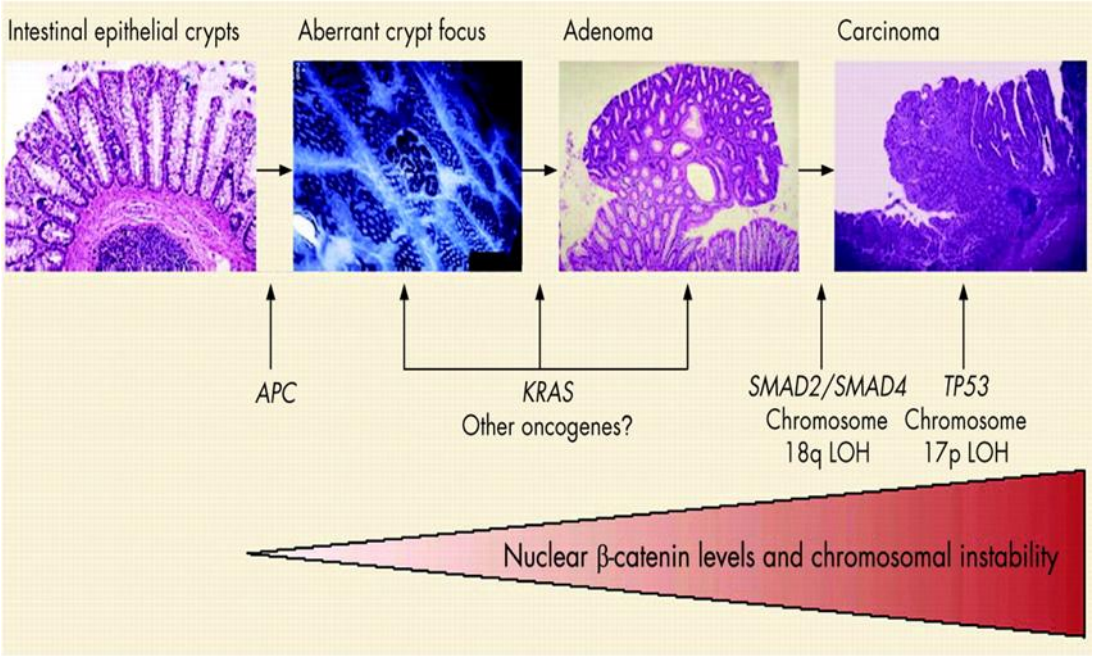
- Is found to be associated with inactivation of DNA mismatch repair proteins and 15–20% of sporadic CRCs are found to have microsatellite instability (MSI-H).
- In sporadic colon carcinoma, MSI is caused by loss of expression of MLH1, secondary to MLH1 promoter methylation, while in hereditary nonpolyposis colon cancer / HNPCC inherited mutation of a repair gene is the cause.

- Sporadic MSI-H cancers are usually poorly differentiated, mucinous and more proximally located than HNPCC tumors
- BRAF mutations can also be associated with the microsatellite instability pathway

(C) CpG island methylator pathway (CIMP)

- CIMP appear to be an independent predictor for microsatellite status.One third of CpG island methylator phenotype underlies microsatellite instability
- It is also strongly associated with BRAF mutation.

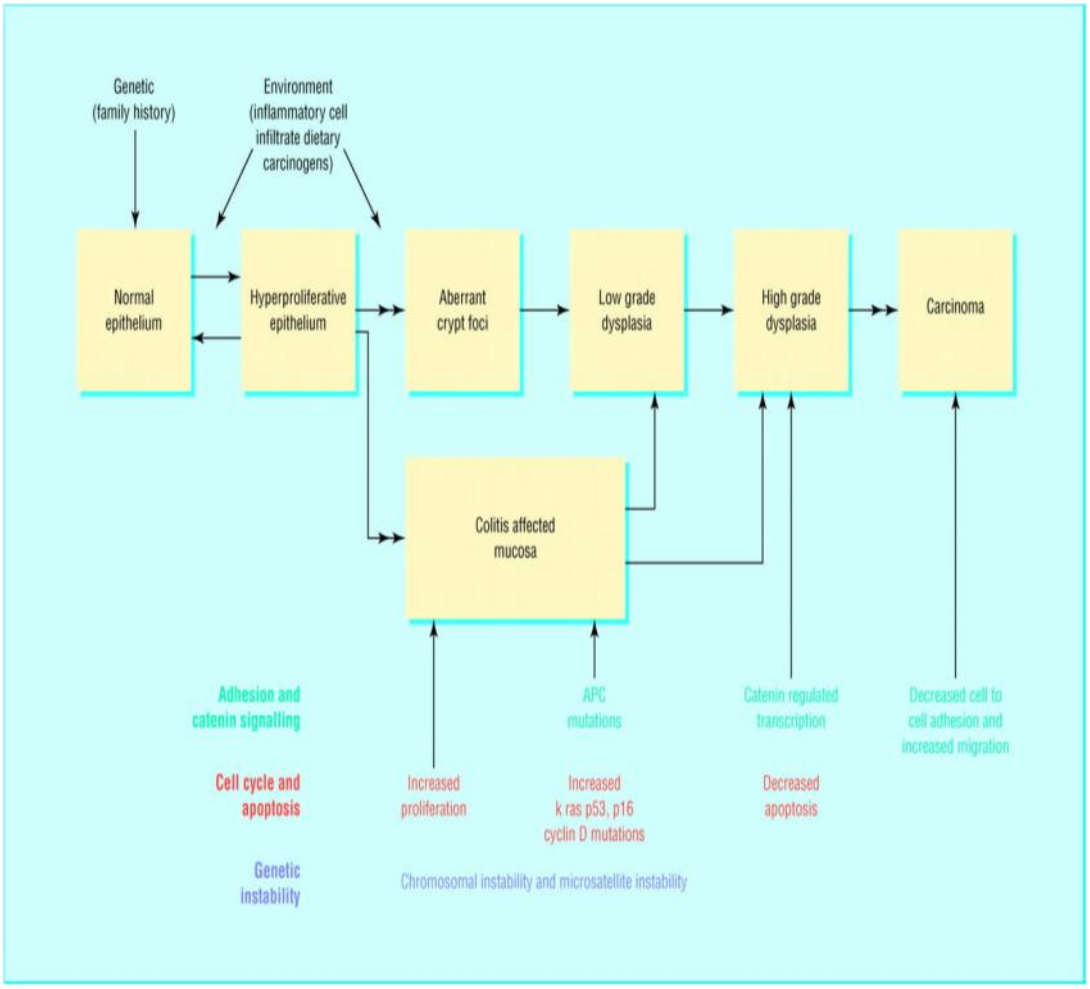
Figure 4 : Formation of adenoma from aberrant crypt focus to development of carcinoma.



Serrated Adenoma Pathway:

- Historically, hyperplastic polyps were usually notconsidered a precursor of carcinoma
- More recent studies have demonstrated that certain types of hyperplastic or serrated polypsmay give rise to cancer
- Sessile serrated adenomas are commonly found to have BRAF mutations in about 78 % of the cases. Few cases have K-ras mutations amounting to about 11%, in contrast to hyperplastic polyps, showing frequent K-ras mutations(80 %)in comparison to BRAF mutations (20%)
- MLH1 promoter methylation can also be found in serrated polyps, suggesting that they give rise to sporadic colorectal carcinoma with MSI.
- Smoking and estrogen withdrawal may be associated with serrated pathway carcinoma.

Figure 5 : Summary of all the mutations that occur



Staging of colon cancers

1. AJCC staging of colon cancers:

TX Primary tumor cannot be assessed

T0 No evidence of primary tumor

Tis Carcinoma in situ: intraepithelial or invasion oflamina propria*

T1 Tumor invades submucosa

T2 Tumor invades muscularis propria

T3 Tumor invades through the muscularis propriainto the subserosa, or into non-peritonealizedpericolic or perirectal tissues

T4 Tumor directly invades other organs orstructures, and/or perforates visceralperitoneum.

Regional lymph node status

NX Regional lymph nodes cannot be assessed

N0 No regional lymph node metastases

N1 Metastases in 1 to 3 regional nodes

N2 Metastases in 4 or more regional nodes

Metastases

MX Distant metastases cannot be assessed

M0 No distant metastases

M1 Distant metastases

Residual tumor assesment

RX Presence of residual tumor cannot be assessed

R0 No residual tumor

R1 Microscopic residual tumor

R2 Macroscopic residual tumor[39]

Table 4: Staging of colon cancers[40]

American Joint Committee on Cancer	*Dukes Staging*	*Modified Astler-Coller Classification*
Stage 0: Tis, N0, M0	A	A
Stage I: T1, N0, M0 or T2, N0, M0	A	A/B_1
Stage IIA: T3, N0, M0	B	B_2
Stage IIB: T4, N0, M0	B	B_2
Stage IIIA: T1-2, N1, M0	C	C_1
Stage IIIB: T3-4, N1, M0	C	C_2
Stage IIIC: T any, N2, M0	C	C_1/C_2
Stage IV: T any, N any, M1	D	D

2.**Dukes staging** :

- Dukes' A: Invasion into but not through the bowel wall.
- Dukes' B: Invasion through the bowel wall but not involving lymph nodes
- Dukes' C: Involvement of lymph nodes
- Dukes' D: Widespread metastases

3.**Astler Coller staging :**

Stage A: Limited to mucosa

- Stage B1: Extending into muscularis propria but not penetrating through it; nodes not involved
- Stage B2: Penetrating through muscularis propria; nodes not involved
- Stage C1: Extending into muscularis propria but not penetrating through it. Nodes involved
- Stage C2: Penetrating through muscularis propria. Nodes involved
- Stage D: Distant metastatic spread

While reporting colorectal cancer , following parameters should be examined and reported :

- Location of tumor
- Size and configuration of the cancer
- Status of resection margins
- Grade of the cancer and type of cancer (e.g., typical, mucinous, small cell)
- Status of lymph nodes :
    - Total number of lymph nodes found
    - Number of lymph nodes involved (pNx, 0, 1, 2)
- Depth of penetration, including direct extension into other organs, if present (pT0, 1, 2, 3, 4)
- Status of veins with regard to tumor invasion:
    - Intramural veins
    - Extramural veins (thick walled or thin walled)
- Presence or absence of lymphatic invasion
- Other lesions present (e.g., adenoma, hyperplastic polyp)

Maspin expression in colonic diseases :

Maspin belongs to serine protease inhibitor / non inhibitor superfamily. Its gene is located on chromosome 18 and was first identified in 1994.Maspin expression has been found to be down regulated in breast, prostate, gastric cancers and upregulated in pancreatic, colorectal and gall bladder cancers. Our study is focused on the expression of maspin in colorectal cancers and studying its significance.

Maspin is found to exhibit suppressing activity against tumor growth and metastasis. Hence it is often silenced in tumors. Maspin has been shown to be involved in processes such as cell invasion, angiogenesis and also apoptosis.

Cao et al. had investigated the relationship between chronic inflammatory states and neoplasia in 125 specimens. His cases under study included inflammatory bowel disease (IBD) with different grades of dysplasia and also invasive colorectal cancer.His study led to the discovery that Maspin was paradoxically over-expressed in both active IBD and colitis-associated dysplasia compared to inactive IBD or normal colonic mucosa. This finding suggested that maspin might have

a potential role in disease “flare” and neoplastic progression [41]. Umekita et al. had studied expression of maspin in colorectal adenocarcinomas from 104 patients. His observations concluded that maspin expression significantly correlated with the higher Dukes’ classification, depth of invasion and highgrade tumor budding. These results suggest that the expression of maspin may be associated with the aggressiveness of colorectal adenocarcinomas.[42] Fung et al. examined 450 resected colorectal cancers . His study also concluded that maspin has a stronger expression in high-grade tumors [43].

Again Dietmaier et al. investigated maspin expression in 172 primary stage III colon cancers . His study showed significant treatment benefit from 5-FU-based chemotherapy in patients with Maspin expression in primary tumors. These data couldbe useful, if confirmed in a prospective study, to select patients to receive 5-FU treatment or an alternative (non-5-FU based) adjuvant therapy .[44]

Hence the present study will be verifying these statements and attempting to find a relationship between maspin expression and the prognosis of colorectal cancers.

Immunohistochemistry:

Immunohistochemistry involves two disciplines – immunology and histology.

Immunohistochemistry is used to determine expression of particular antigen and its microanatomic location in the tissue. IHC uses antibodies to distinguish antigenic differences between the cells.These differences can specifically identify the lineage of cell populations and define biologically distinct population of cells within the same lineage.

Antigen retrieval technique was introduced by Shi and associates in 1991. It's a simple method that involves heating paraffin sections to a high temperature before IHC staining.The use of antibody in IHC depends on sensitivity and specificity of antigen antibody reaction and the hybridoma technique provides limitless source of highly specific antibodies.

Detection systems :

Antibodies are labeled or flagged by some method to permit visualization – these include fluorescent substances, enzymes forming colored reaction with suitable substrate (light microscopy) or heavy metals (electron microscopy).

Methods of IHC

Direct conjugate labeled antibody method

Antibody is attached with a label by chemical means and directly applied to tissue sections. It is a rapid and easy procedure and involves detection of multiple antigens which require separate incubation with specific antibodies.

Indirect sandwich method

Enzymes are labeled with secondary antibody which is produced against primary antibody.The advantages are increased versatality, high working dilution of primary antibody and easy preparation of secondary antibodies against a primary antibody of different species.

Unlabelled antibody methods

Enzyme bridge technique

Here the labeled moiety is linked to the antigen solely by immunologic binding.

Peroxidase antiperoxidase method :

The principle of the PAP method is similar to that of the enzyme bridge method. The acronym PAP denotes the peroxidase

antiperoxidase reagent that is composed of antibody against horseradish peroxidase antigen in the form of an immune complex which is small and stable.Available evidence suggests that this immune complex is made up of two antibody molecules and three horseradish peroxidase molecules. The PAP reagent and the primary antibody must be derived from the same species (or it can also be from closely related species with common antigenic determinants),whereas the bridge or linking antibody is derived from a second species and has specificity against the primary antibody.

Avidin biotin technique

The high affinity between biotin and avidin is used in this technique; Biotin binds to the primary antibody and avidin binds to the enzyme thus attaching it to the biotinylated antibody. Disadvantage of this procedure is the presence of endogeneous biotin activity that produces non specific background staining.

Avidin biotin conjugate procedure

Here the primary antibody is added followed by biotinylated secondary antibody and next preformed complexes of avidin and biotin horse radish peroxidase conjugate.

Biotin Streptavidin system

Streptavidin is used in place of avidin. Streptavidin complexes are more stable compared to avidin.

Immunogold silver technique

This is used in ultrastructural immunolocalisation. Gold particles are enhanced by addition of several layers of silver.

Polymeric method

This technique allows a large number of enzyme molecules to be bound to a secondary antibody via dextran backbone. The advantages of this technique are increased sensitivity in identifying the antigen , minimal non specific background staining and decreased number of assay steps.

Alkaline phosphatase and antialkaline phosphatase method

The principles are same as that of PAP method.

Tissue fixation, Processing and antigen retrieval techniques

Tissues for IHC undergo fixation , dehydration and Paraffin embedding.

Fixation

This is a critical step as preservation of morphology is essential for interpretation. 10 % neutral buffered formalin is used.It has the following advantages :

1. Good morphological preservation

2. Cheap, easily available, penetrates tissues well and sterilizes them.

3. Carbohydrate antigens are better preserved and does not interfere with the staining process.

The disadvantage of masking antigens during fixation can be overcome by antigen retrieval technique.

Antigen retrieval

The following techniques are used for unmasking of the antigen

1. Proteolytic enzyme digestion method
2. Microwave antigen retrieval
3. Pressure cooker antigen retrieval
4. Microwave and trypsin antigen retrieval technique

MATERIALS AND METHODS

Source of data

A total of70 cases including 10 controls (normal colonic mucosa) of non neoplastic and neoplastic colorectal diseases .The cases included are Non specific colitis, Ulcerative colitis, colorectal adenomas and adenocarcinomas received in the Department of Stanley Medical College from the Department of Surgery in the year 2012-2013.

Inclusion criteria

All patients diagnosed to have chronic non neoplastic and neoplastic colorectal disease**s** diagnosed by biopsies as well as colectomy specimens are included in the study**.**

Method of data collection

All colorectal biopsies,colectomy and hemicolectomy specimens were included in the study. Appropriate tissues were sampled and processed. Sections were cut at 5 microns and stained by H & E technique and examined under the microscope for adequacy and appropriate photomicrographs were taken. All the selected cases were included in the immunohistochemical analysis to study Maspin

expression. Only 70 cases were studied due to the cost effectiveness of the immunohistochemical study.

Method of tissue preparation for IHC

10 % buffered formalin was used to fix the tissues. Then the tissues were processed in various grades of alcolhol and xylol using automated histokinette.Paraffin blocks were prepared and 5 micron thickness sections were cut and stained using H& E. Suitable blocks were chosen for IHC.

Sections for immunohistochemistry were also cut in the semi automated microtome.These sections were 4 microns thick and positively charged slides were used . Sections were subjected to antigen retrieval technique by pressure cooker method using TRIS EDTA (Ph 9) buffer solution and then treated by HRP (horse radish peroxidase) polymer technique.

HRP polymerTechnique

1. The sections were deparrafinised in xylene or xylene substitutes
2. Rehydrated through graded alcohols
3. The slides were then washed in running tap water
4. The antigen retrieval was performed using the appropriate buffer by pressure cooker method.
5. The endogeneous peroxide was blocked using peroxidase block for 5 mins
6. Slides were then washed in 2 changes of TBS buffer for 5 mins each.
7. Slides were then incubated with protein block for 5 mins
8. Then slides were washed in 2 changes of TBS buffer for 5 mins each.
9. Optimally diluted primary antibody was then used to incubate the slides for 60 mins.
10. Then the slides were washed in 2 changes of TBS buffer for 5 mins each.
11. Then incubation with post primary for 30 mins
12. Then the slides were washed in 2 changes of TBS buffer for 5 mins each.
13. Then incubation with novolink polymer for 30 mins

14. Then the slides were washed in 2 changes of TBS buffer for 5 mins each.
15. Then peroxidase activity was developed with DAB working solution.
16. The slides were then rinsed in water, counterstained in hematoxylin, washed in water, dehydrated, cleared and mounted to be examined.

Method of studying Maspin expression

The immunoreactivity to maspin was identified by staining of the cytoplasm. 100 cells were randomly selected and counted from 5 representativefields.

A four tier grading system was used to study the intensity of expression:

1. Negative (-) 0-5%
2. Weakly-positive (+), 6-25%
3. Moderately-positive (++), 26-50%
4. Strongly-positive (+++), 51-100%.

This grading system was used in the study by Zheng et al [45].

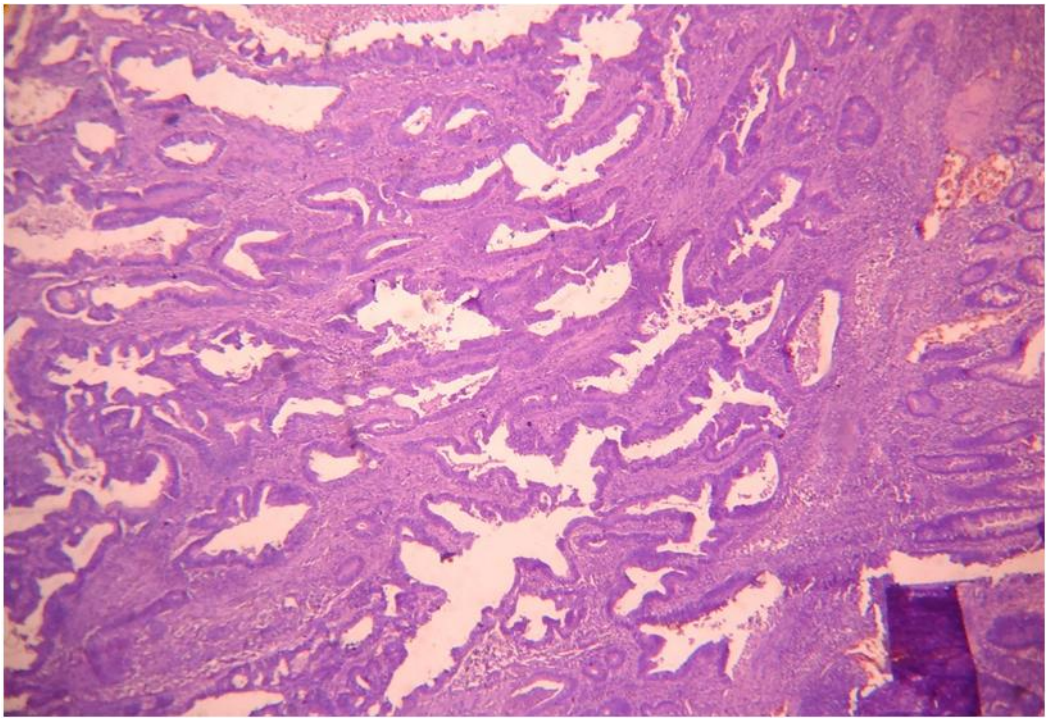


Figure 6 :Colorectal Adenocarcinoma (H & E, 10 X)

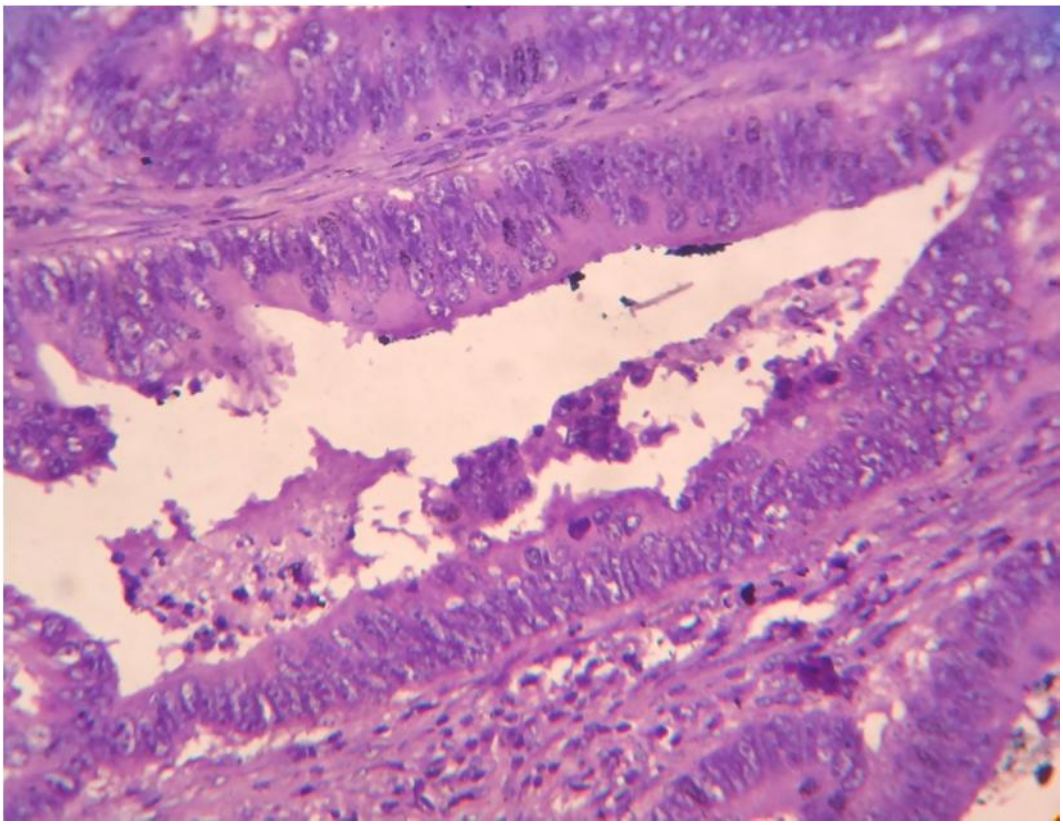


Figure 7 :Colorectal Adenocarcinoma (H & E, 40 X)

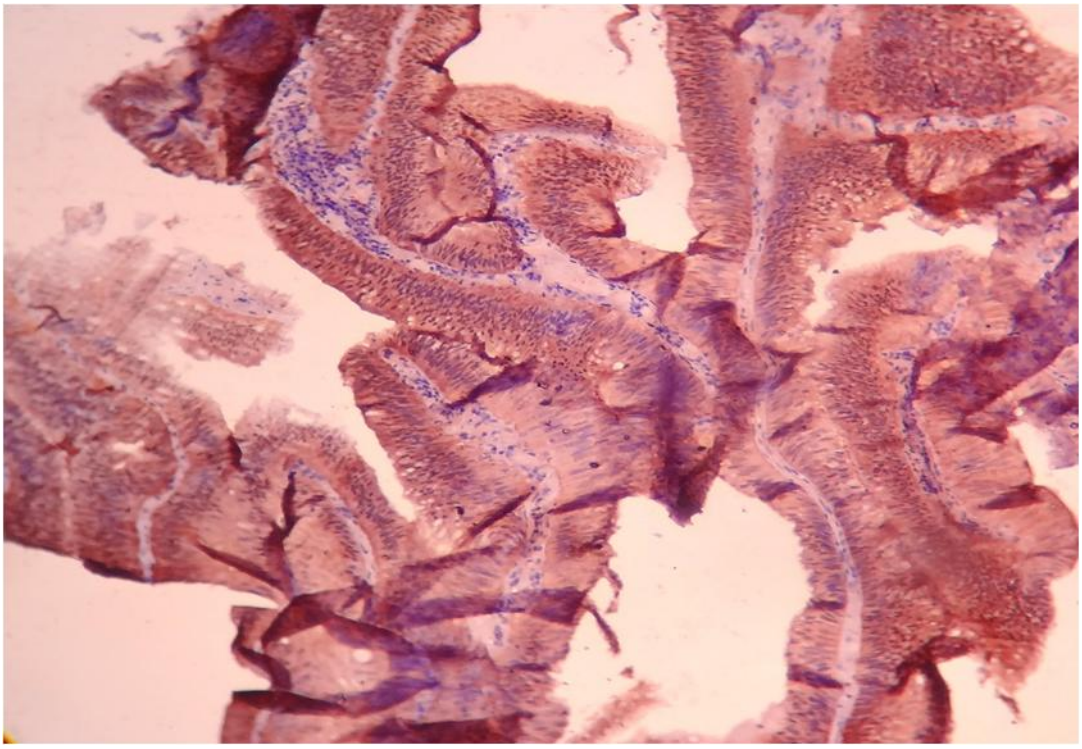


Figure 8 :Maspin expression (3 +) in colorectal adenocarcinoma – (10 X)

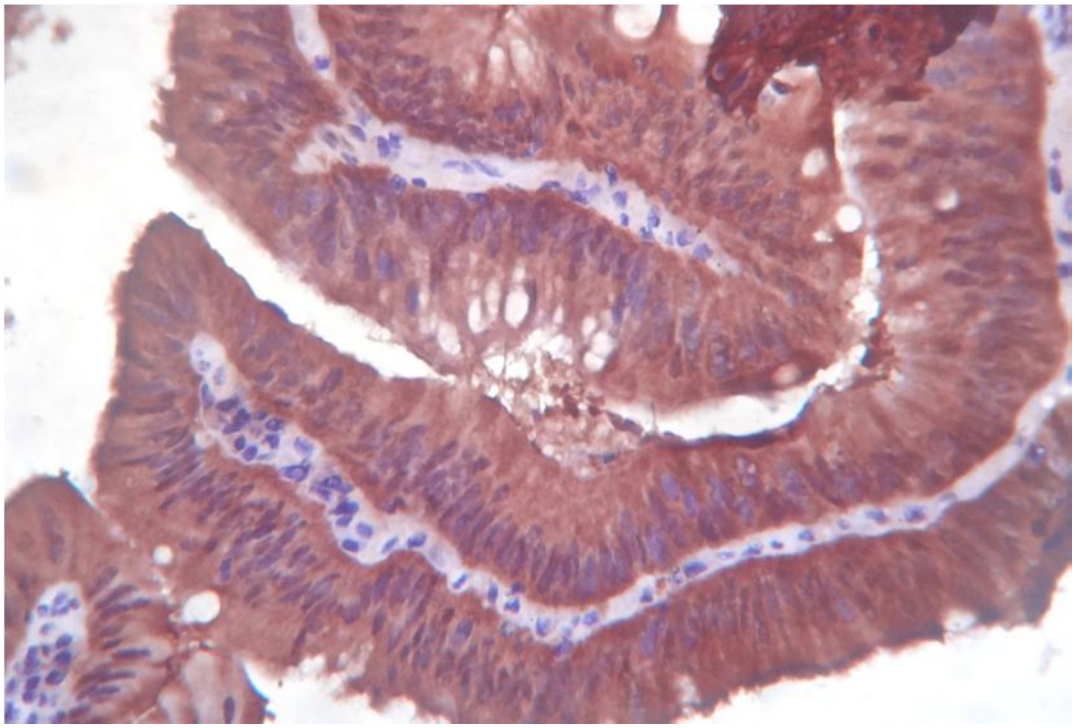


Figure 9 :Maspin Expression (3 +) in colorectal Adenocarcinoma (40 X)

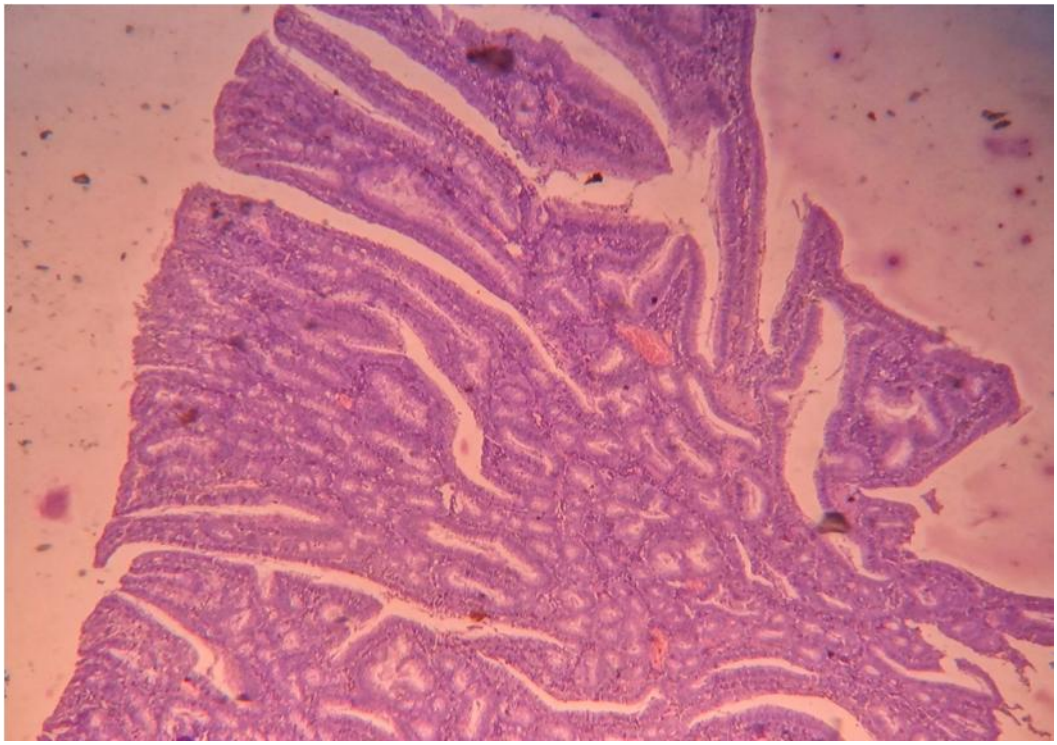


Figure 10 :Adenoma – (H & E, 10 X)

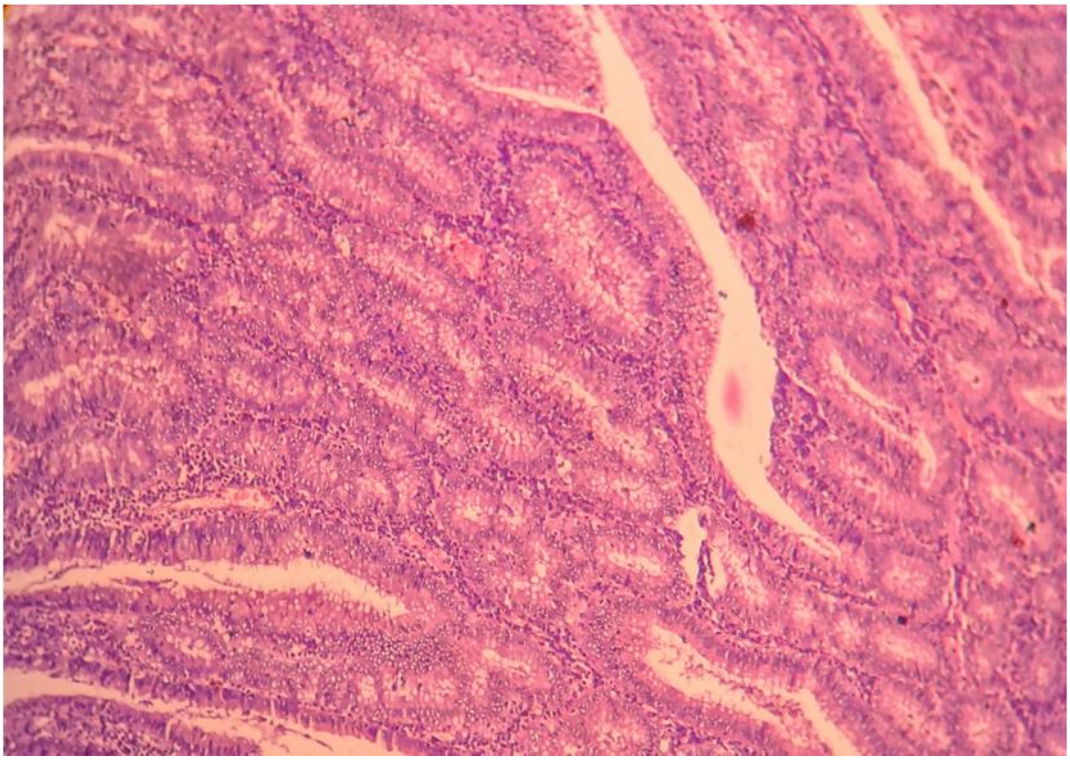


Figure 11 :Adenoma – (H & E, 40 X)

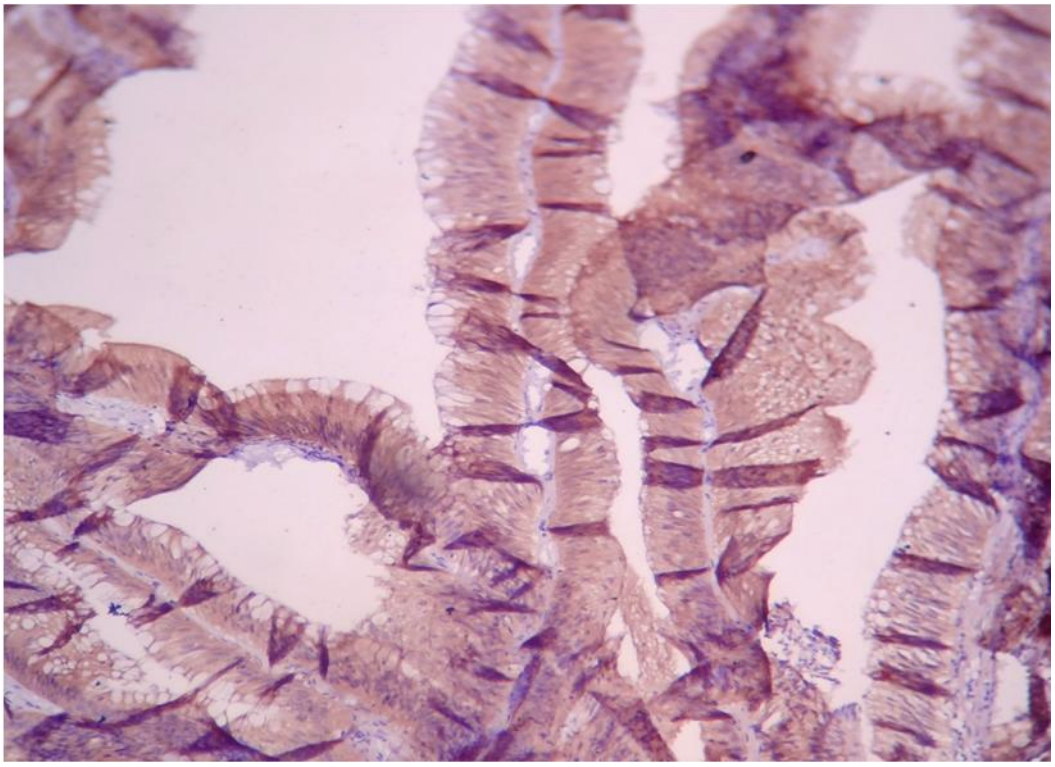


Figure 12 :Maspin Expression (3 +) in Adenoma – (10 X)

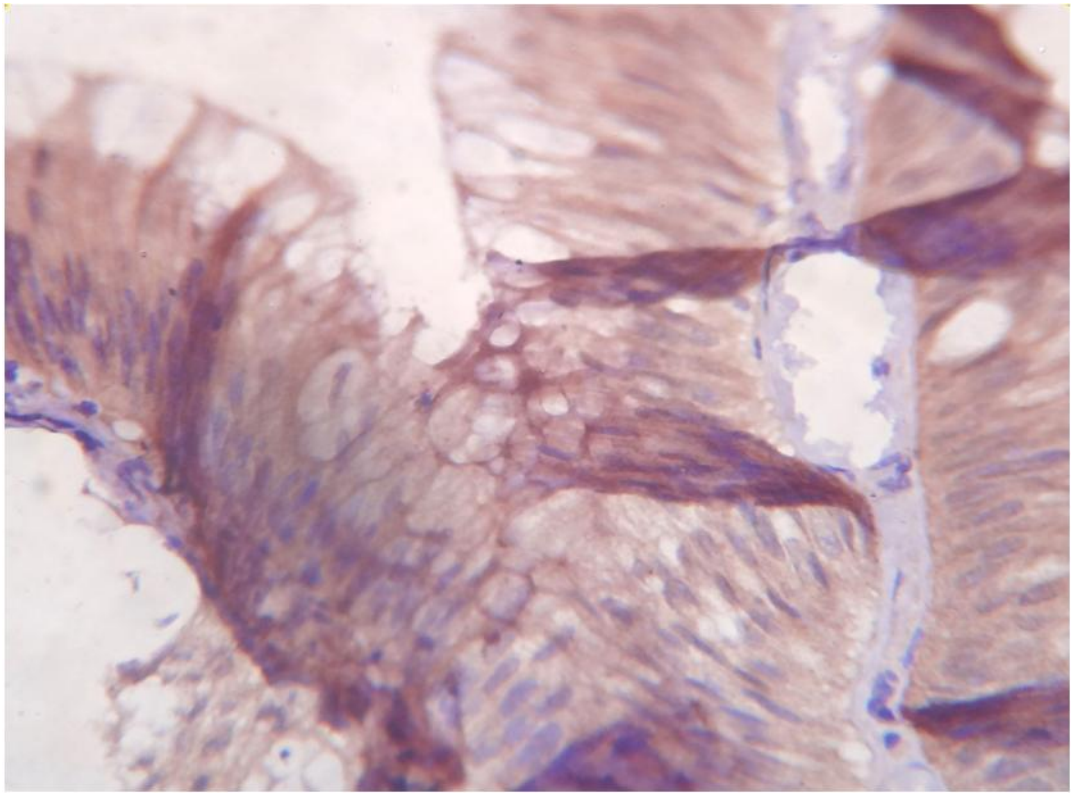


Figure 13 :Maspin Expression (3 +) in Adenoma – (40 X)

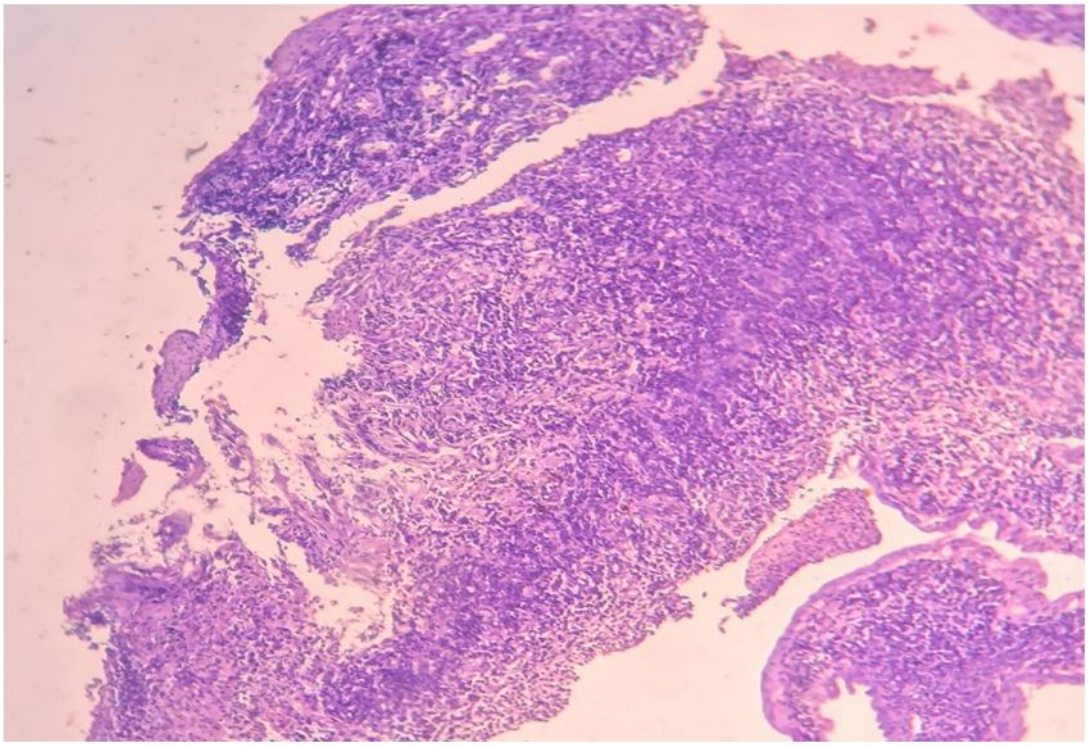


Figure 14 :Ulcerative Colitis (H & E, 10 X)

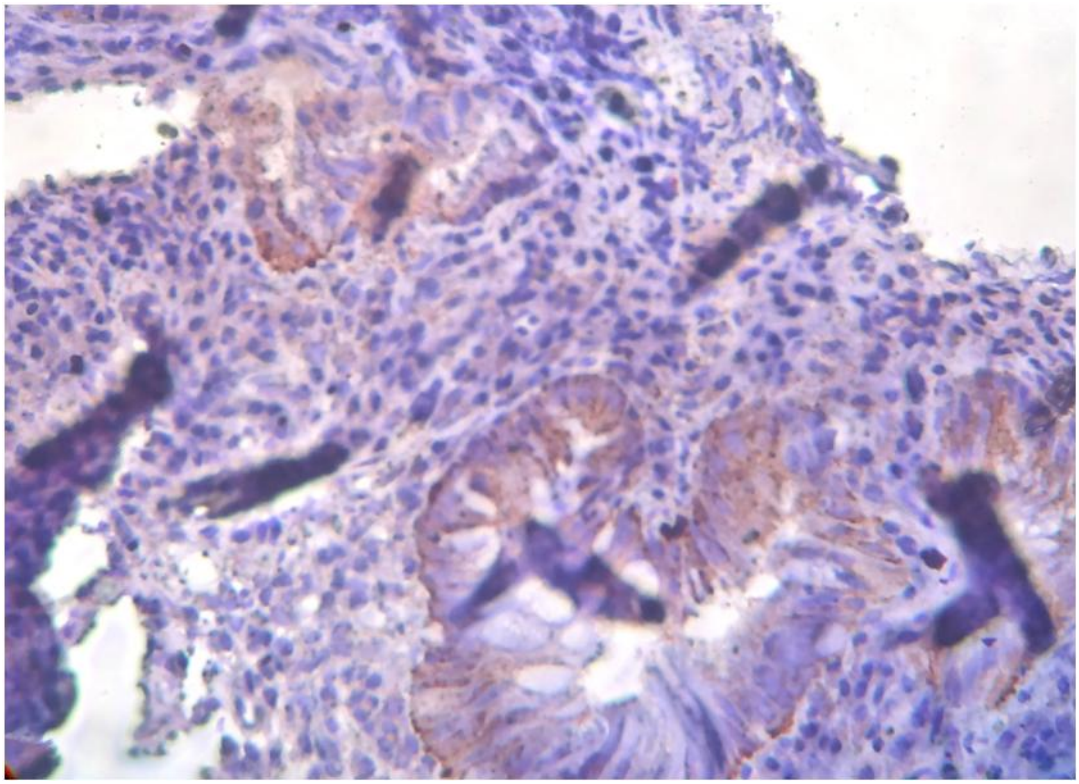


Figure 15 :Maspin Expression (1 +) in Ulcerative colitis (40 X)

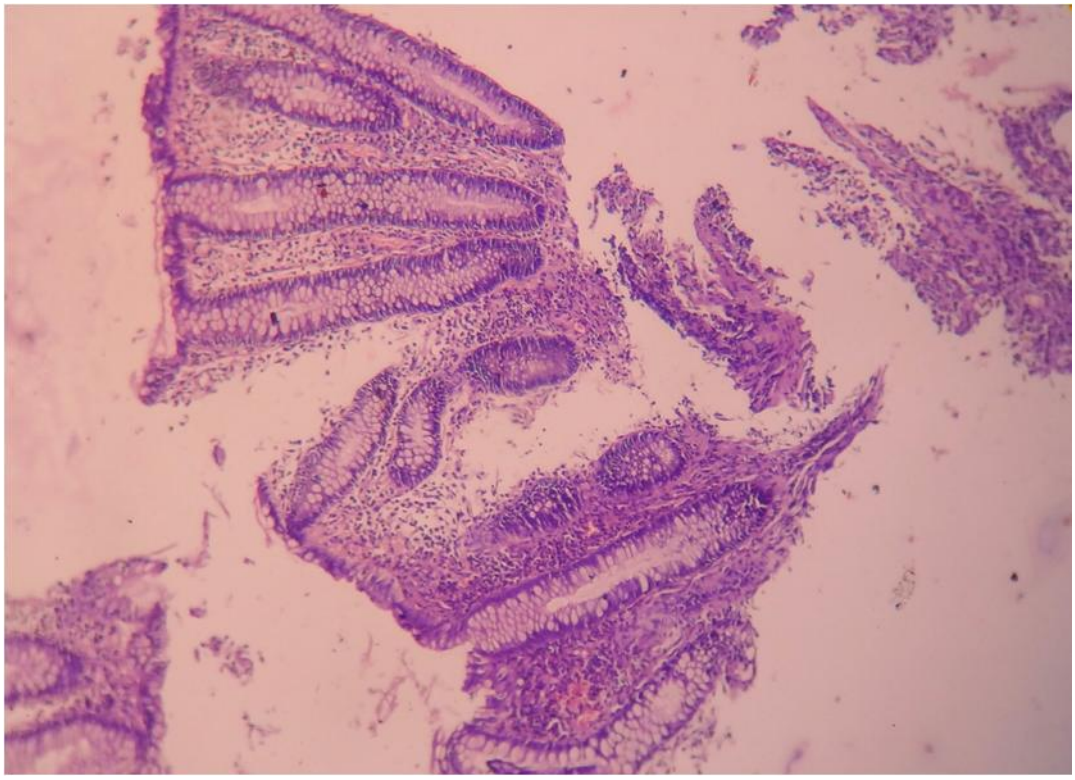


Figure 16 :Non Specific colitis – (H & E, 10 X)

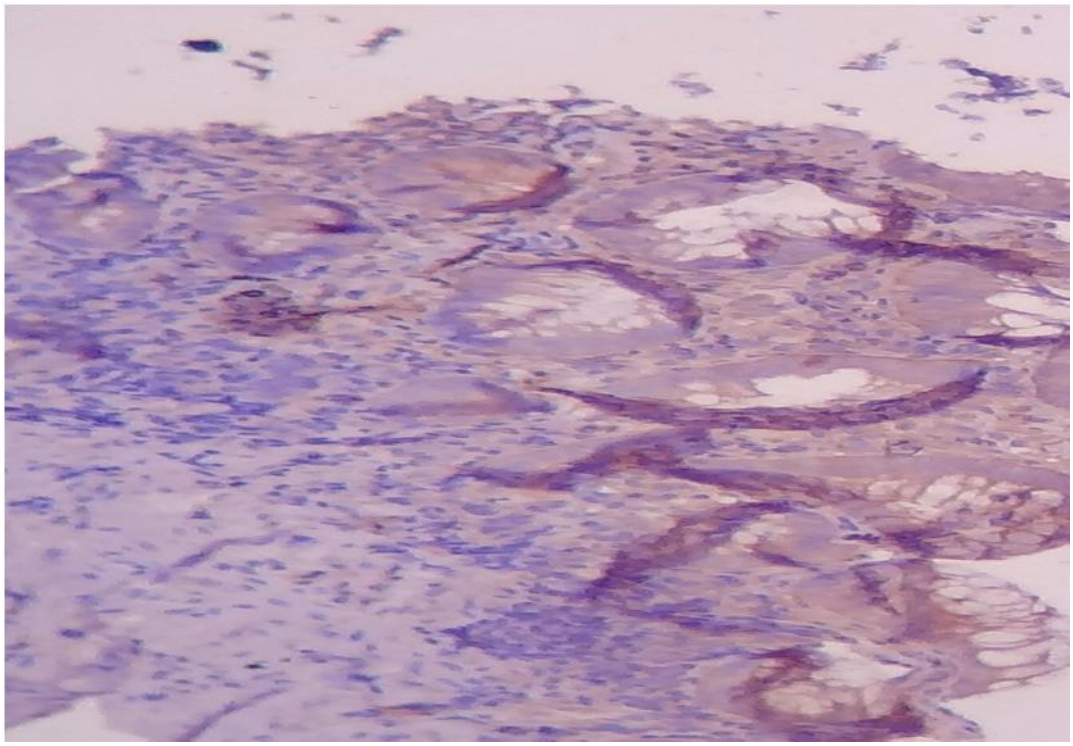


Figure 17 :Maspin Expression (1 +) – Non specific colitis (10 X)

OBSERVATION AND RESULTS

70 cases were selected representing different colorectal neoplastic and non neoplastic conditions. The following indicates the distribution of the cases.

Table 5

Distribution of Cases

Case		Number
1.	Normal colonic mucosa	**10**
2.	Non specific colitis	**10**
3.	Ulcerative colitis	**10**
4.	Adenocarcinoma including 4 cases ofAdenoma with malignant transformation	**40**

Immunohistochemical analysis and results

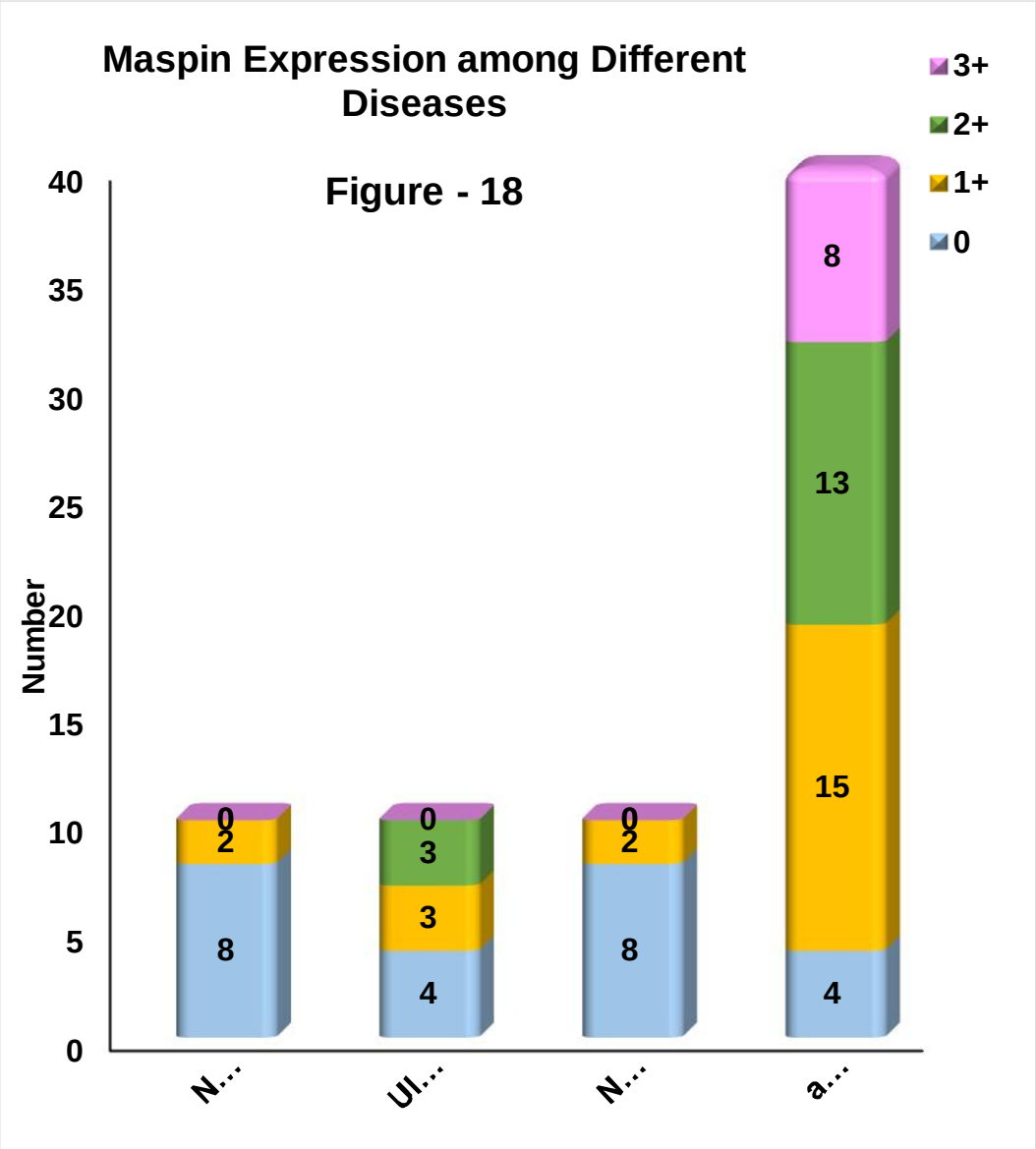
Immunohistochemistry was done for all the above 70 cases and the following results were tabulated.

Table 6

Significance of maspin expression in non neoplastic and neoplastic colorectal diseases

Diseases	n	0	1+	2+	3+	Percentage positivity
Normal colonic mucosa	10	8	2	0	0	20%
Ulcerative colitis	10	4	3	3	0	60%
Non specific colitis	10	8	2	0	0	20%
Adenocarcinoma	40	4	15	13	8	90%

Chi-Square Test	P-Value
Fisher's Exact Chi-Square test	<0.001



Thus <u>significant difference</u> in expression of maspin was found from normal colonic mucosa to ulcerative colitis to adenocarcinoma.

This is similar to the findings in the study by Payne et al[46] which showed a significant difference in maspin expression in neoplastic and non neoplastic colorectal diseases.

Table 7

Study by Payne et al

Mean maspin expression in non-neoplastic colonic mucosa of colon resections from patients with and without colonic neoplasia: comparison to mean maspin expression of adenomas and adenocarcinomas

Control colonic mucosa from patients without colonic neoplasia (n = 9)	Non-neoplastic colonic mucosa from patients with colonic neoplasia (n = 10)	Adenomas/Adenocarcinomas (n = 12)
0.65 ± 0.20	0.66 ± 0.20	2.17 ± 0.83
Student's *t*-test	$P = 0.93$[1]	$P = 0.00002$[2] $P = 0.00004$[3]

[1]**Notes:** Non-significant difference in mean maspin expression compared with control colonic mucosa;
[2]significant difference in maspin expression compared with nonneoplastic colonic mucosa from patients with colonic neoplasia;
[3]significant difference in maspin expression compared with control colonic mucosa.

The inference from this table shows that there is a significant difference in the expression of maspin in different conditions with it being the highest in colorectal cancers compared to other conditions .More number of cases showed +3 expression as we moved from ulcerative colitis to adenoma to carcinoma. This is comparable with the findings of Payne et al in their study of maspin which is **a deoxycholate-inducible, anti-apoptotic stress-response protein. Its expression is modified in colon carcinogenesis.This is also consistent with the study of Cao et al[41].They had assessed 25 cases of colorectal carcinoma showing 88% positivity of maspin and 51 cases of active chronic IBD showing 92 % positivity.**

Table 8

Comparison table with other studies

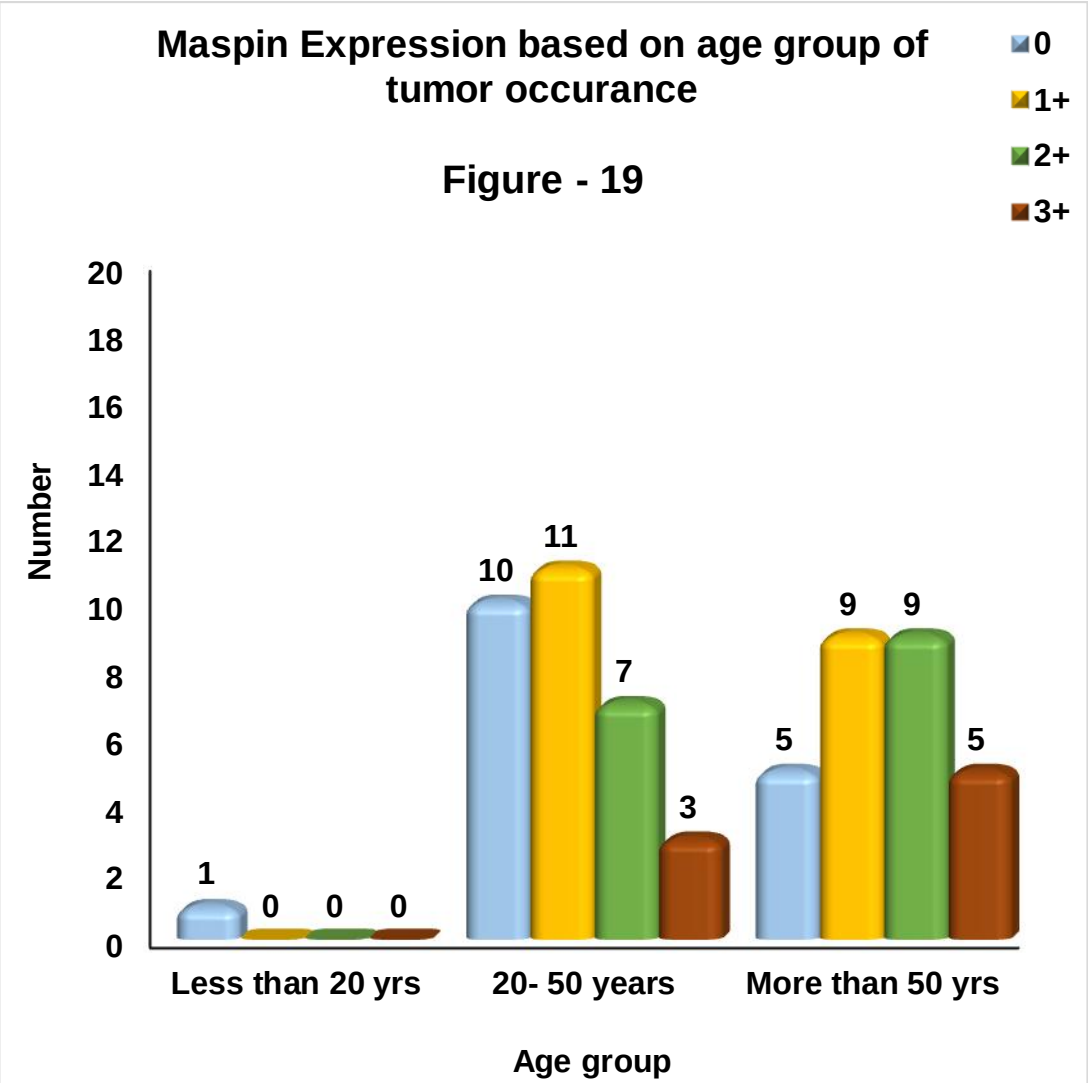
Study	Year	Inference –p value
Payne et al	2011	less than 0.05
Cao et al	2005	Less than 0.05
Song et al [47]	2002	Less than 0.05
Fung et al	2010	Less than 0.05
Present study	2012-2013	Less than 0.05

Table 9

Relationship between maspin expression and age of the patient

Age	Number	0	1+	2+	3+
Less than 20 yrs	1	1	0	0	0
20- 50 years	31	10	11	7	3
More than 50 yrs	28	5	9	9	5

Chi-Square Test	P-Value
Fisher's Exact Chi-Square test	0.516



This shows that no significant difference was present between maspin expression and the age of the patient which is also an important prognostic factor .According to certain studies, young and very old age groups were at high risk for aggressive course of the disease[48,49,50,51].But our finding was found to be consistent with the study by Pasz- Walczak G et al[52] that maspin expression did not co-relate with age of the patient.

Table 10

Comparison table with other studies.

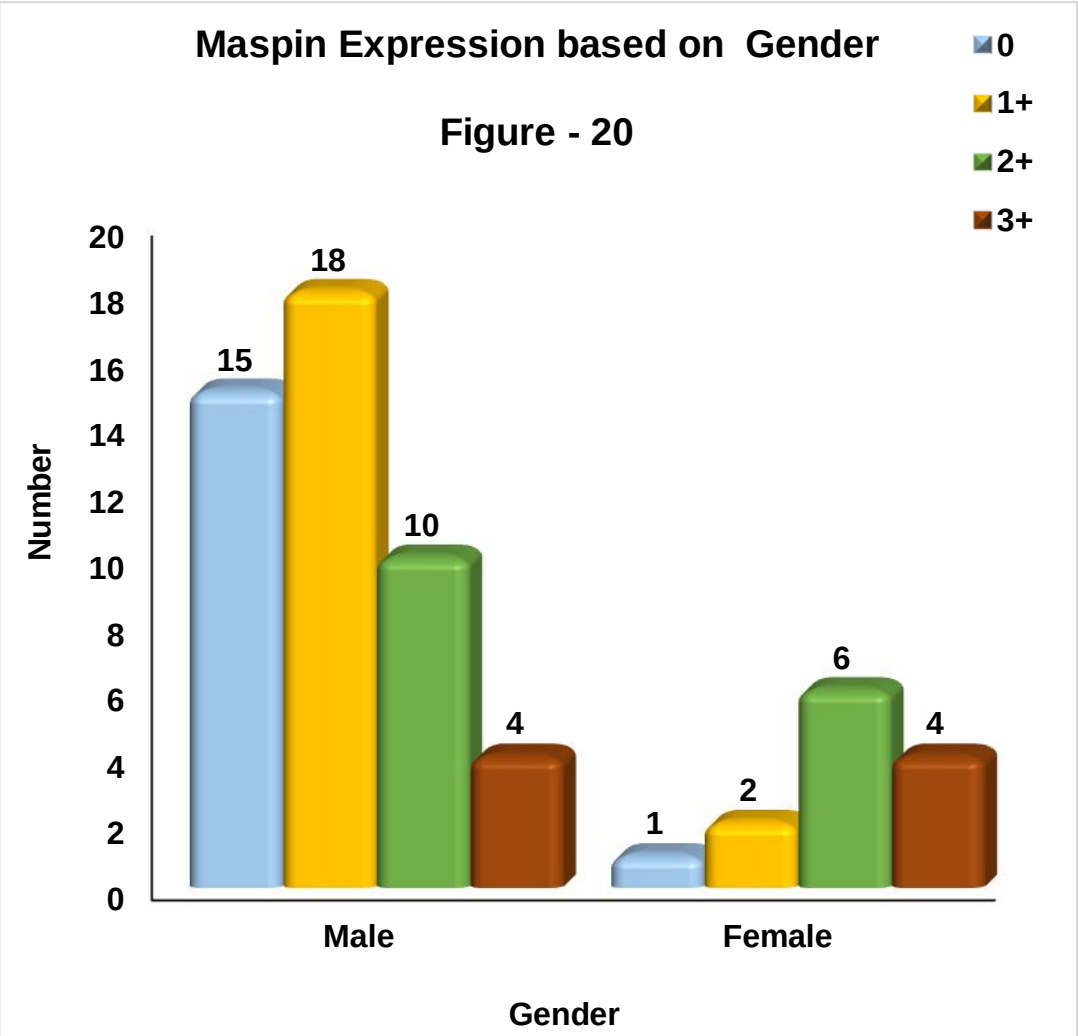
Study	Year	Inference
1. Pasz- Walczak G et al	2010	More than 0.05
2. Present study	2012-2013	More than 0.05

Table 11

Relationship between maspin expression and sex of the patient

Sex	Number	0	1+	2+	3+
Male	47	15	18	10	4
Female	13	1	2	6	4

Chi-Square Test	P-Value
Fisher's Exact Chi-Square test	0.018



This table shows that the expression of maspin **significantly correlated** with the sex of the patient. According to the study by Griffin et al[48] males are at higher risk for an aggressive course of the diseases compared to females.Our study was comparable with the following study by boltze et al[53] in which significant difference was found in the expression of maspin and the sex of the patient, thus showing

increased expression in males .

Table 12

Comparison table with other studies

Study	Year	Inference –P value
1. Boltze et al	2005	Less than 0.05
2. Present study	2012-2013	Less than 0.05

Table 13

Site distribution of neoplastic and non neoplastic diseases

Diseases	number	Right sided lesions	Left sided lesions
Non specific colitis	10	7	3
Ulcerative colitis	10	4	6
Adenocarcinoma	40	10	30

This table shows that left sided lesions are more common compared to right sided lesions in adenocarcinomas. This is comparable with the following studies.

Table 14

Comparison table with other studies for neoplastic diseases[54,55,56]

Study	Year	Inference – most common site
Peedikayil et al	2009	Rectum
Chattar cora et al	1976- 1995	Rectum
Qing SH et al	2003	Rectum
Present study	2012-2013	Rectum

Table 15

Expression of maspin in right sided and left sided lesions in both neoplastic and non neoplastic diseases

Site	Number	0	1+	2+	3+
Right side	21	7	9	3	2
Left side	39	9	11	13	6

Chi-Square Test	P-Value
Fisher's Exact Chi-Square test	0.306

This table shows that there is <u>no significant difference</u> between maspin expression and the site of the disease.

This is contradication with the studies by Snoeren et al[57] and Fung et al which stated that right sided lesions had a higher expression compared to left sided lesions.

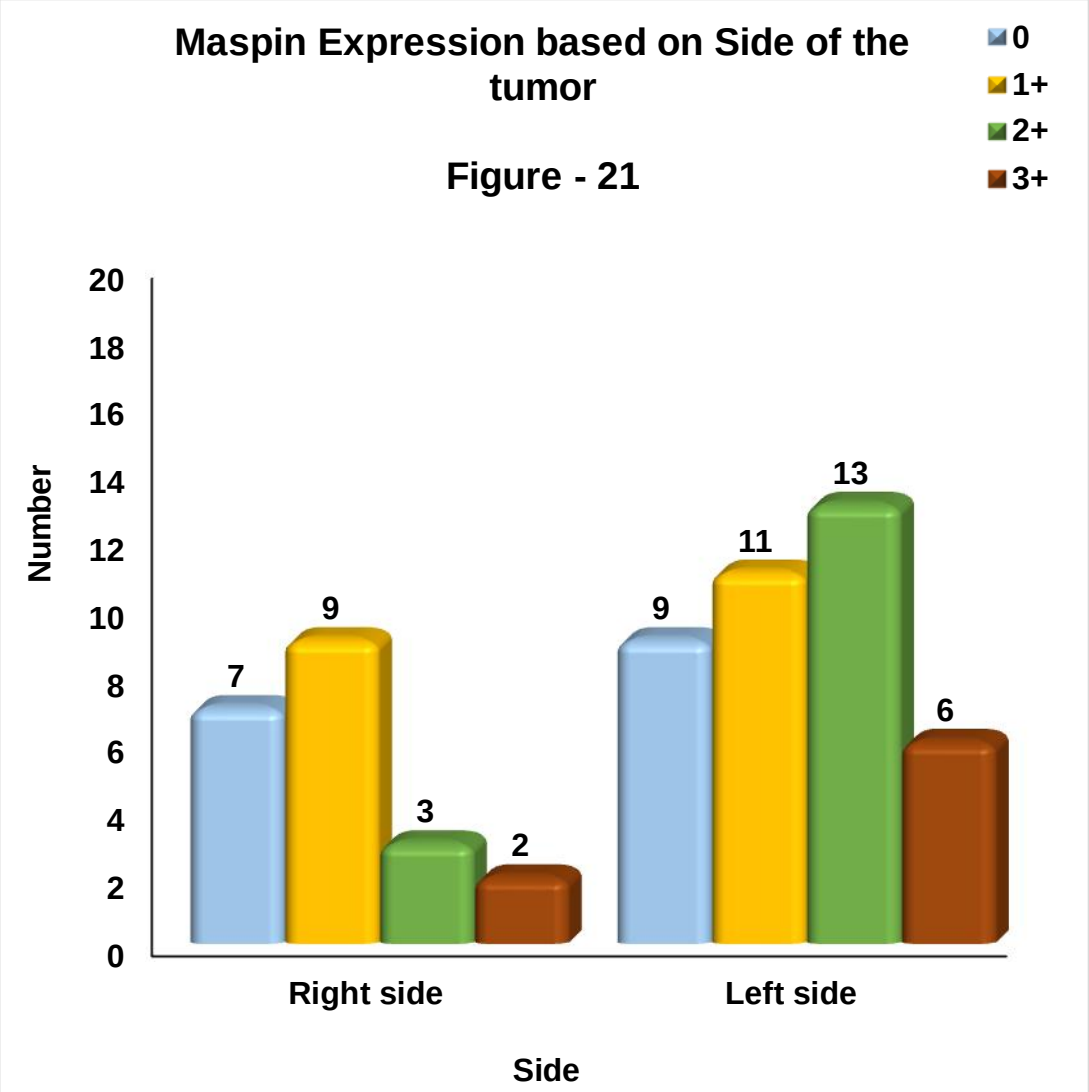


Table 16

Comparison table with other studies

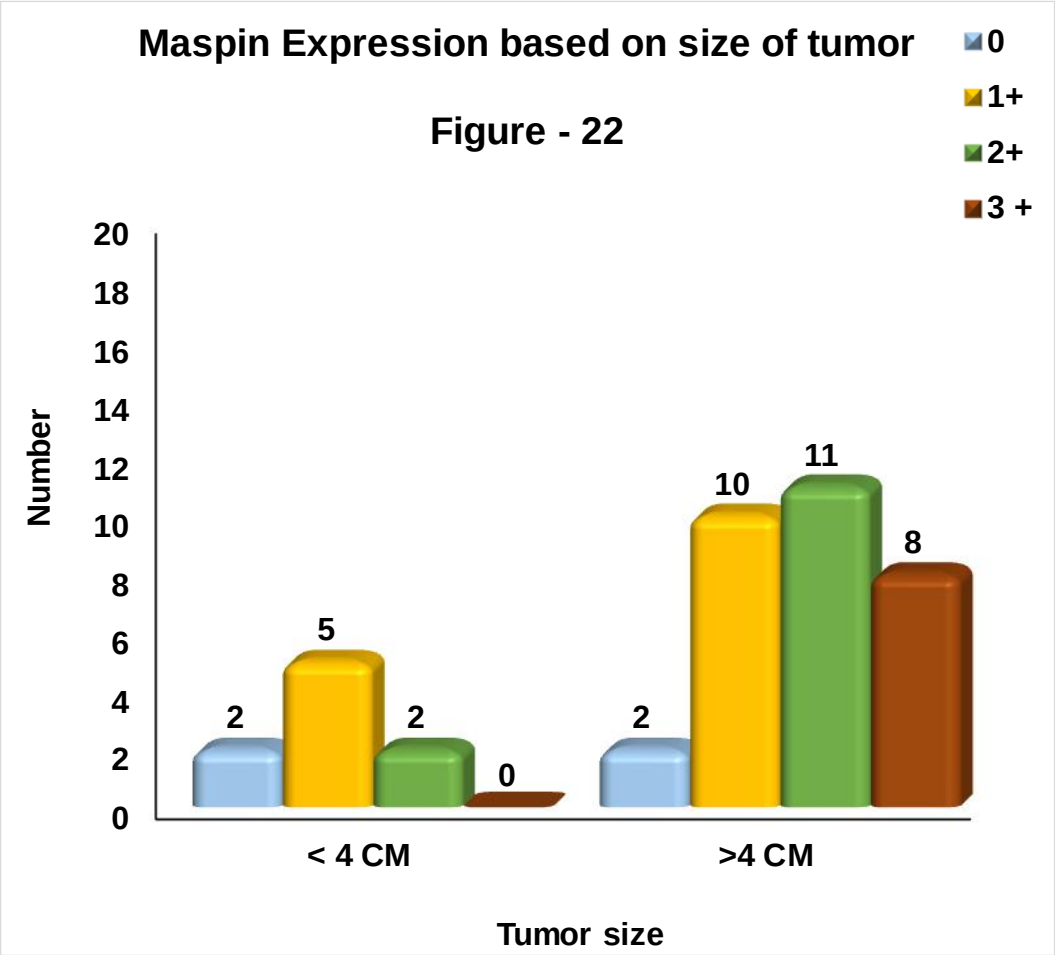
Study	Year	P Value
1. Snoeren et al	2012	Less than 0.05
2. Fung et al	2010	Less than 0.05
3. Present study	2012-2013	More than 0.05

Table 17

Significance of maspin expression based on tumor size

Tumor size	n	0	1+	2+	3 +
< 4 CM	9	2	5	2	0
>4 CM	31	2	10	11	8

Chi-Square Test	P-Value
Fisher's Exact Chi-Square test	0.110



This table shows that there is no significance between expression of maspin and the tumor size. This was comparable with the following studies.

The following table is a study by Zheng et al.

Table 18

Significance of Maspin expression and tumor size by Zheng et al.

Tumor size	n	0	1+	2+	3+	Percentage positivity	P value
Less than 4	47	1	7	6	33	98	> 0.05
More than 4 cms	72	5	14	15	38	93	

This table shows that there is no significance between the tumor size and the expression of maspin. This is comparable with the findings of Zheng et al

Table 19

Comparison table with other studies

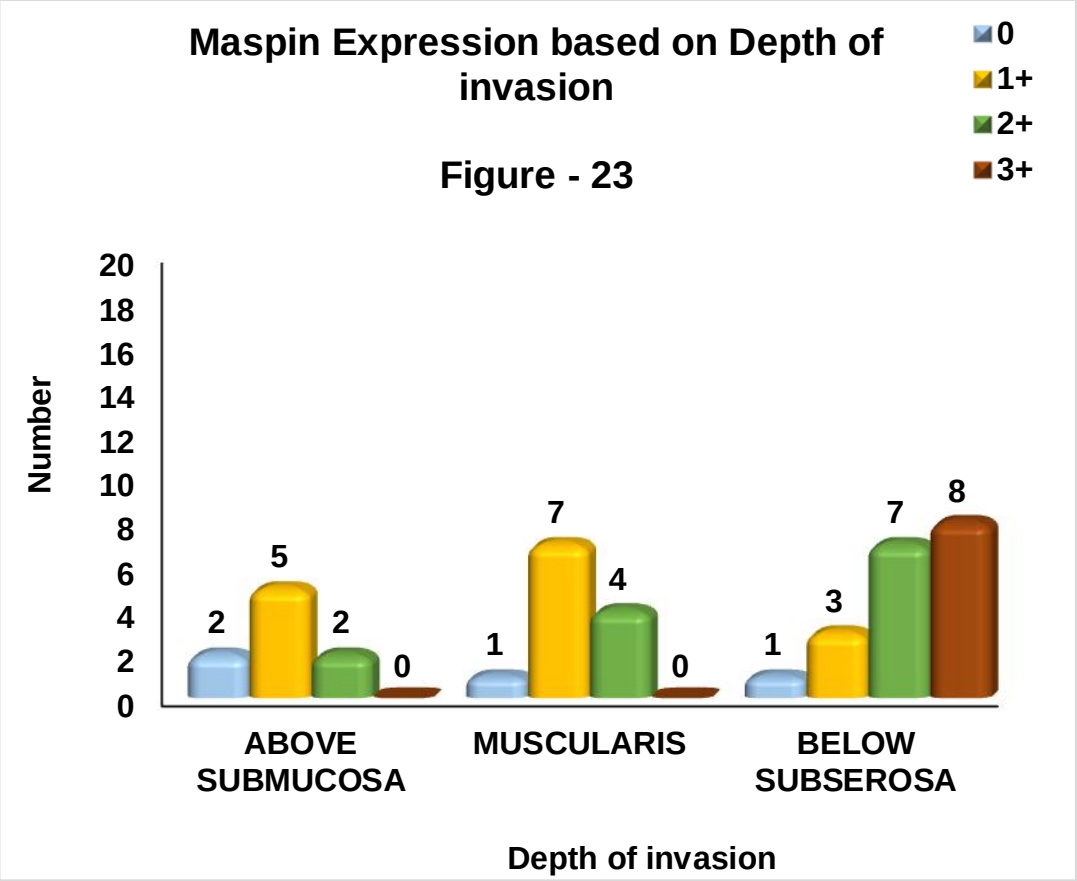
Study	Year and inference
Zheng et al	2007,P >0.05
Present study	2012-2013, P>0.05

Table 20

Significance of maspin expression and depth of invasion

DEPTH OF INVASION	N	0	1+	2+	3+
ABOVE SUBMUCOSA	9	2	5	2	0
MUSCULARIS	12	1	7	4	0
BELOW SUBSEROSA	19	1	3	7	8

Chi-Square Test	P-Value
Fisher's Exact Chi-Square test	0.010



This table shows that there is a significant difference in the expression of maspin as the depth of invasion increases.This is consistent with the findings of Umekita et al. This study showed significant difference in the expression of maspin as the depth of invasion varies. Thus the conclusion drawn from this table is that **increased expression of maspin was associated with increasing depth of invasion of the tumor.**

Table 21

Study by Umekita et al

Maspin expression	**Positive**	**Negative**
• **Depth of invasion**		
• **T1, T2**	**3**	**17**
• **T3, T4**	**66**	**18**
• P value – less than 0.0001		

Table 22

Comparison table with other Studies

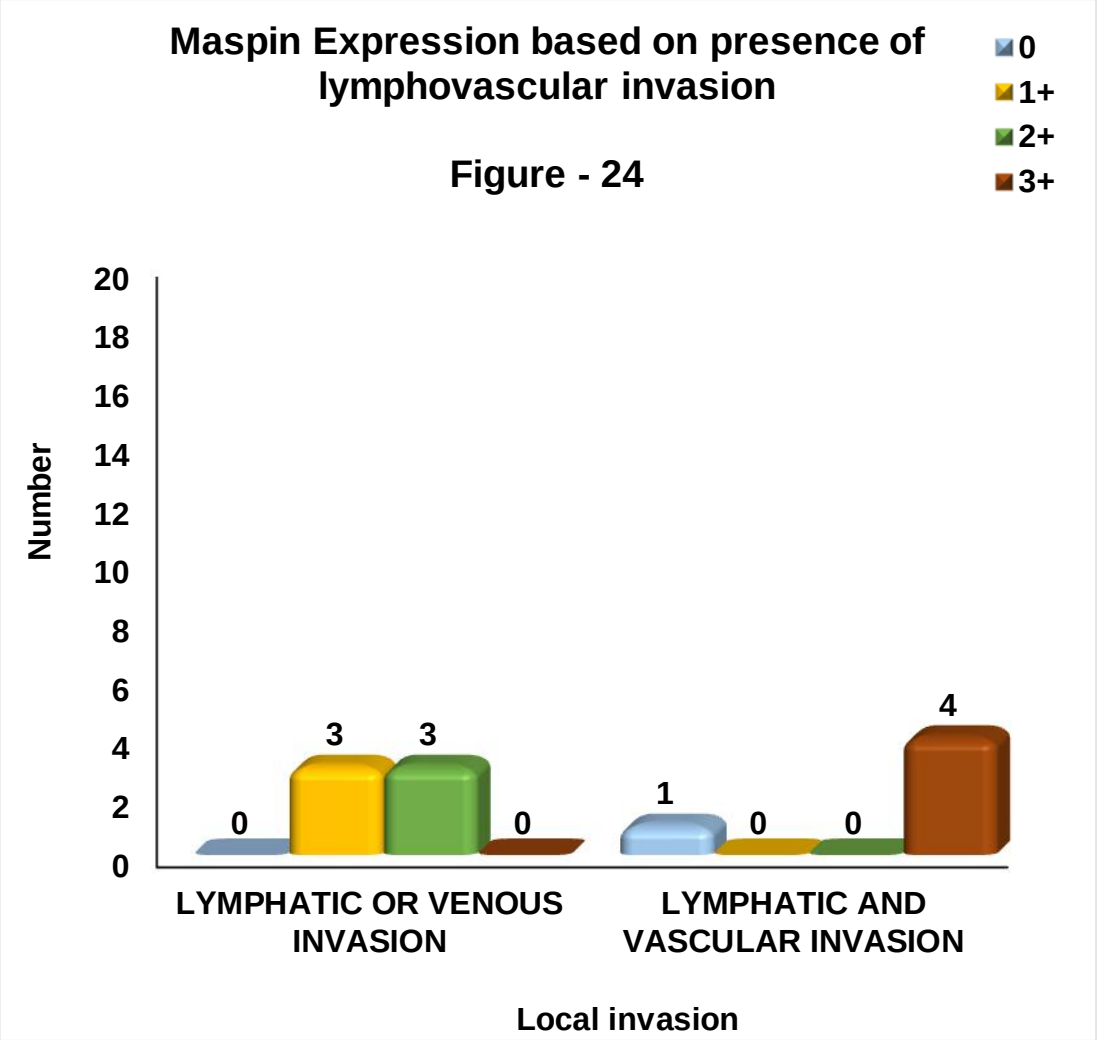
Study	**Year**	**Inference**
Umekita et al	2006	P<0.05
Pasz Walczak et al	2010	P<0.05
Present study	2012-2013	P <0.05

Table 23

Significance of maspin expression and lymphovascular invasion

Lymphovascular INVASION	N	0	1+	2+	3+
LYMPHATIC OR VENOUS INVASION	6	0	3	3	0
LYMPHATIC AND VASCULAR INVASION	5	1	0	0	4

Chi-Square Test	P-Value
Fisher's Exact Chi-Square test	0.002



This table shows that there is a significant difference in the expression of maspin as there is variation in the lymphovascular invasion. This is in contradiction with the studies by Zheng et al and Umekita et al. But in the study by Pasz-Walczak G et al increased maspin expression was associated with vascular invasion. This is comparable with our study showing **increased maspin expression associated with presence of lymphatic and vascular invasion.**

Table 24

Study by Zheng et al

- Local invasion via vessels>0.05

n	0	1+	2+	3+	percentage
– (negative)	30	1	6	4	19	97
• Lymphatic or venous invasion	34	2	4	7	21	94
• Lymphatic & venous invasion	55	3	11	10	31	95

Table 25

Study by Umekita et al

• **Vascular invasion**	**positive**	**negative**
• **present**	**45**	**19**
• **absent**	**24**	**16**

- **P value – 0.278**

Table 26

Comparison table with other studies

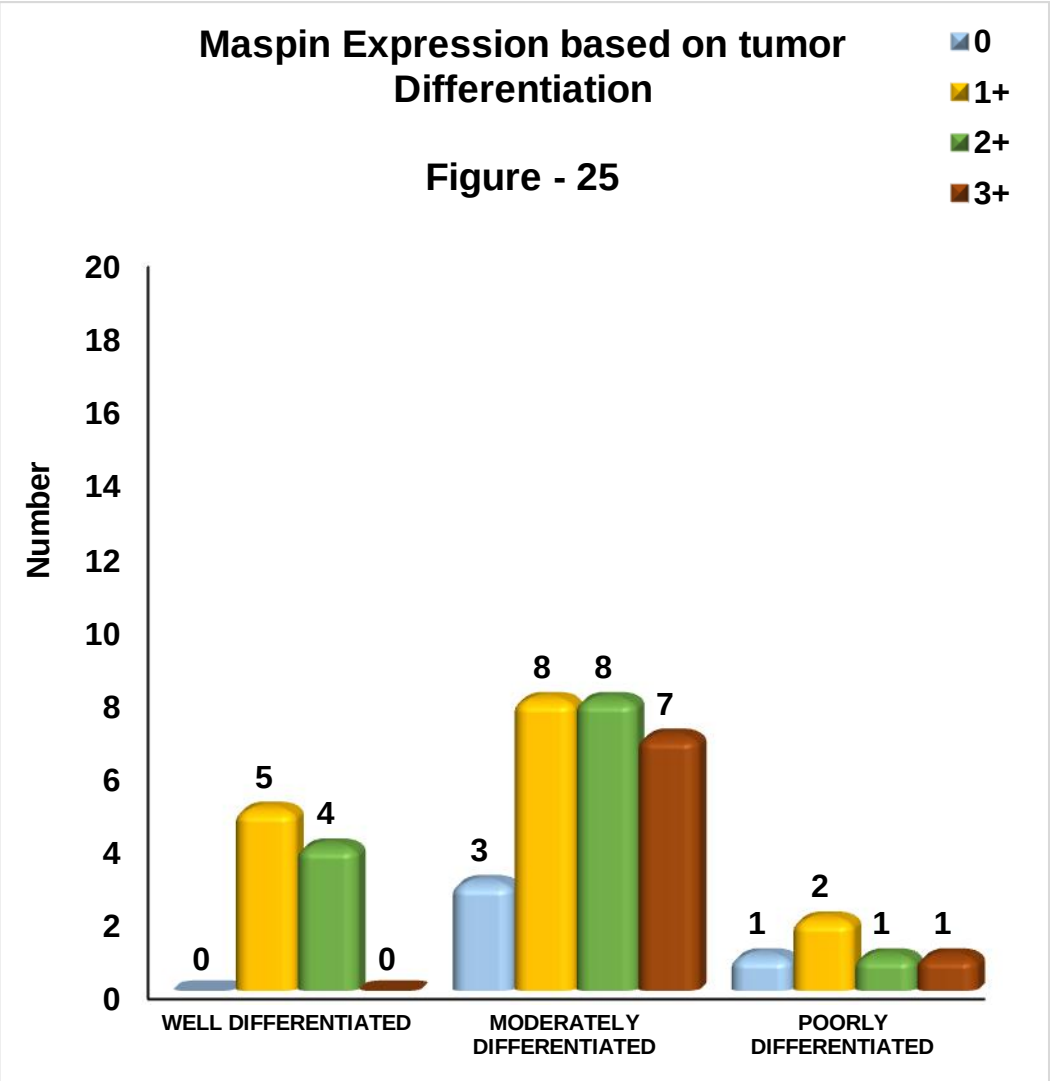
Study	Year	Inference
Zheng et al	2007	P >0.05
Umekita et al	2006	P>0.05
Pasz walczak et al	2010	P<0.05
Present study	2012-2013	P<0.05

Table 27

Significance of maspin expression based on differentiation of tumor

DIFFERENTIATION	N	0	1+	2+	3+
WELL DIFFERENTIATED	9	0	5	4	0
MODERATELY DIFFERENTIATED	26	3	8	8	7
POORLY DIFFERENTIATED	5	1	2	1	1

Chi-Square Test	P-Value
Fisher's Exact Chi-Square test	0.423



This table shows that there is no significant difference in expression of maspin with variation in tumor differentiation .

Table 28

Significance of maspin expression in the presence of liver metastasis

LIVER METASTASIS	N	0	1+	2+	3+
PRESENT	2	1	0	0	1
ABSENT	38	3	15	13	8

Chi-Square Test	P-Value
Fisher's Exact Chi-Square test	0.095

This table shows that there is no significant difference in the expression of maspin in the presence of liver metastasis.

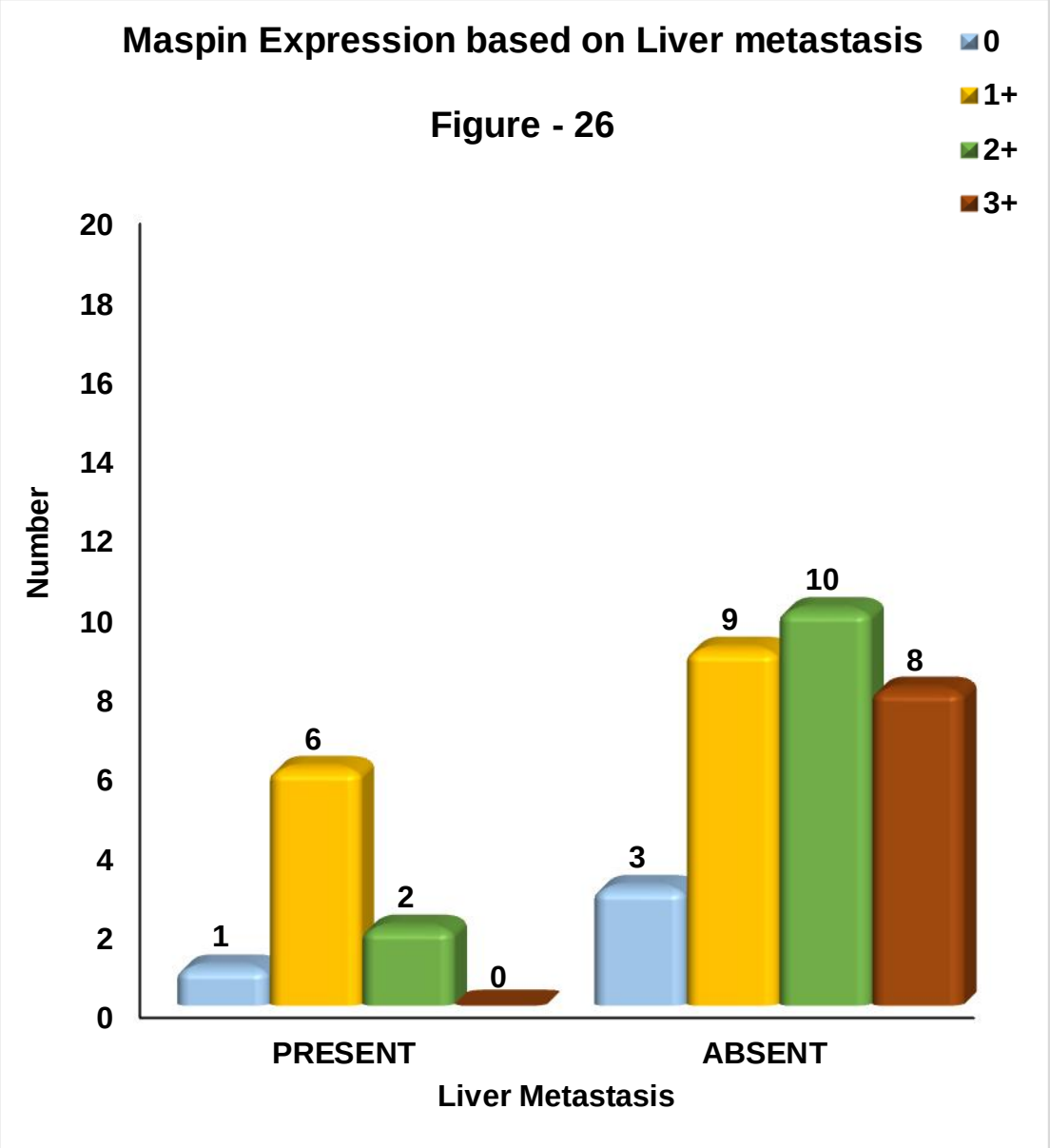
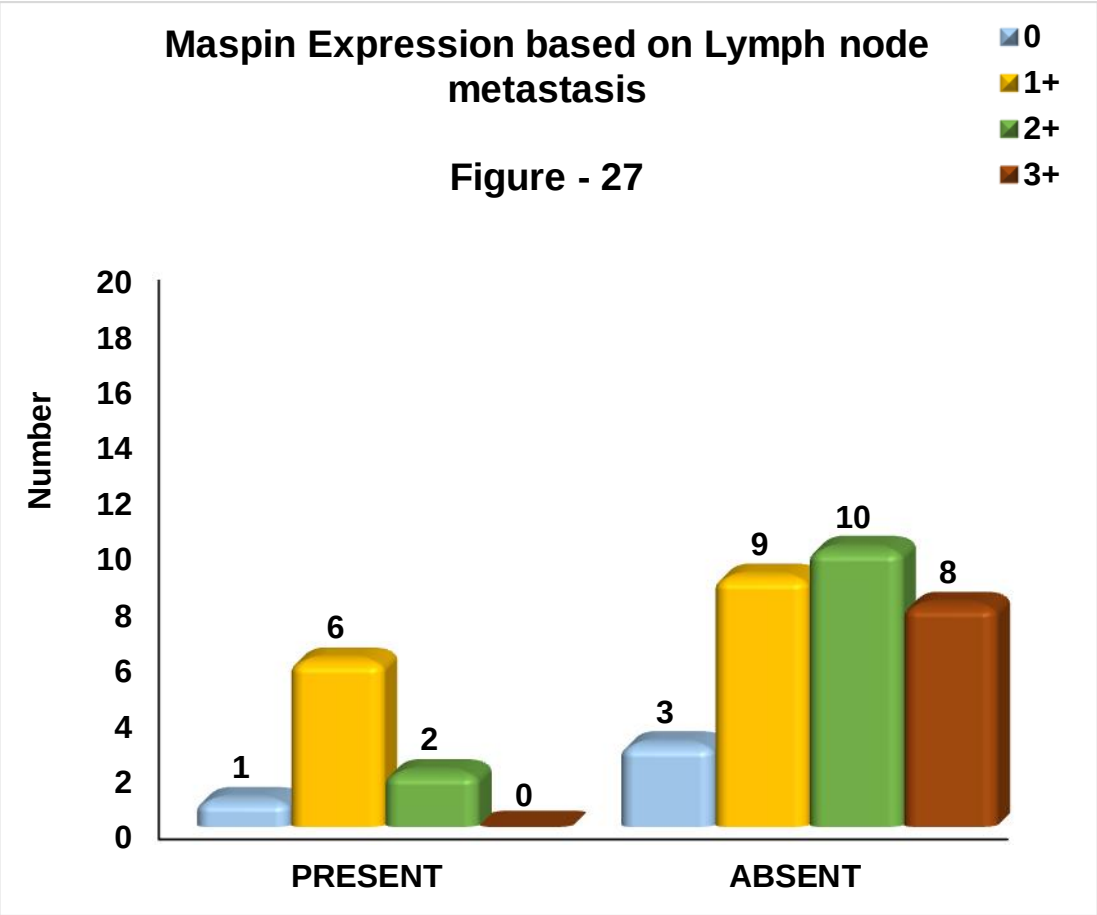


Table 29

Significance of maspin expression in the presence of lymph node metastasis

LYMPH NODE METASTASIS	N	0	1+	2+	3+
PRESENT	9	1	6	2	0
ABSENT	31	3	9	10	8

Chi-Square Test	P-Value
Fisher's Exact Chi-Square test	0.144



This table shows that there is no significant difference in the expression of maspin in the presence or absence of lymph node metastasis.These findings were consistent with the studies by Umekita et al where no significant co-relation was observed between maspin expression and liver metastasis, lymph node metastasis or tumor differentiation.Whereas in Zheng et al significant correlation was found between maspin expression and liver metastasis whereas that of tumor differentiation and lymph node metastasis the results were same as our study.

Table 30

Study by Zheng et al

⦿ Lymph node metastasis >0.05	n	0	1+	2+	3+
⦿ –	49	2	10	9	28
⦿ +	70	4	11	12	43
⦿ Liver metastasis <0.05					
⦿ –	96	11	13	14	58
⦿ +	33	5	8	7	13
⦿ Differentiation >0.05					
⦿ Well-differentiated	68	2	11	13	42
⦿ Moderately-					
⦿ differentiated	45	3	10	5	27
⦿ Poorly-differentiated	6	1	0	3	2	8

Table 31

Study by Umekita et al

• **Maspin expression**	**Positive**	**Negativep value**	
• **Lymph node metastases**			
• **present**	**34**	**11**	
• **absent**	**35**	**200.287**	
Differentiation			
⦿ well	41	27	0.198
⦿ moderate	24	7	
⦿ Poor	4	1	

Table 32

Comparison table with other studies

Study	Year	Parameter	Inference
Zheng et al	**2007**	**1.Lymph node metastasis**	**1.P value >0.05**
		2.Differentiation	**2. >0.05**
		3.Liver metastasis	**3.<o.o5**
Fung et al	**2010**	**1.Tumor differentiation**	**1.<0.05**
Snoeren et al	**2013**	**1.Tumor differentiation**	**1.<0.05**
Umekita et al	**2006**	**1.Lymph node metastasis**	**1. >0.05**
		2.Tumor differentiation	**2. >0.05**
Jiang Tao Ni et al[58]	**2009**	**1.Tumor differentiation**	**1.>0.05**
Pasz Walczak et al	**2010**	**1.Tumor differentiation**	**1.<0.05**
Present study	**2012-**	**1.Lymph node metastasis**	**1. >0.05**
	2013	**2.Tumor differentiation**	**2. >0.05**
		3.Liver metastasis	**3. >0.05**

Table 33

Maspin expression and survival rates

Maspin expression	Number	Survival rates
0	**16**	**93.75%**
1+	**20**	**85%**
2+	**16**	**81%**
3+	**8**	**50%**

Chi-Square Test	Value	P-Value
Trend Chi-Square	5.491	0.019

This table shows that there is a significant difference in the expression of maspin and survival rates of patients with both neoplastic and non neoplastic colorectal diseases.

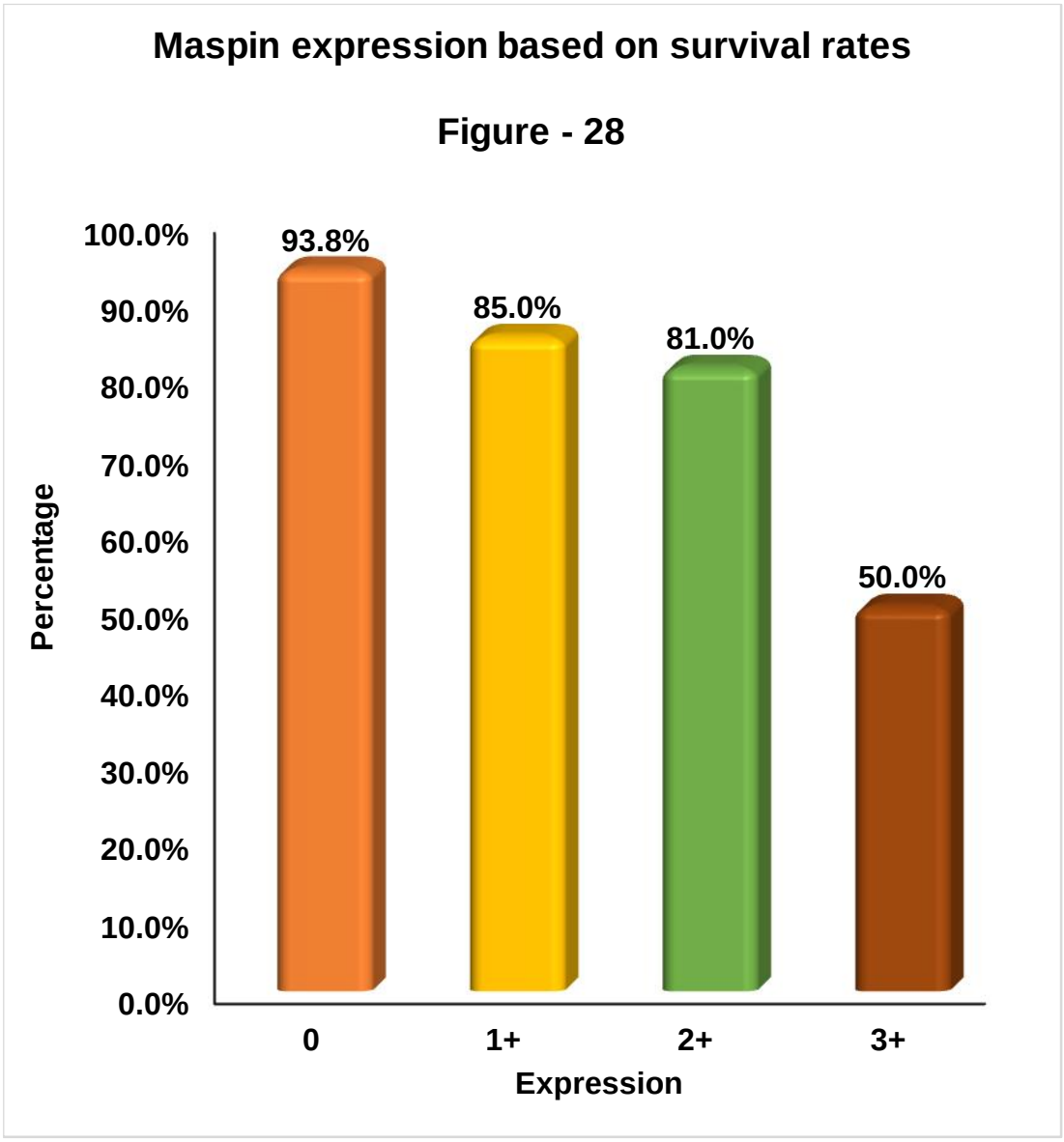


Table 34

Comparison table with other studies

Study	Year	Inference – survival rates and maspin expression
Gurzu et al	**2013**	**Poor survival**
Pasz walczak et al	**2010**	**No association with survival**
Present study	**2012-13**	**Poor survival**

Our study is thus comparable with the study by Gurzu et al which shows that higher the expression of maspin, poor is the survival.

DISCUSSION

Colorectal carcinomas are common in people with western type of diet. Hence the incidence is more in individuals adopting a western lifestyle and slowly the occurrence in increasing in the Asian population compared to the western population. [59]

In this study, several clinicopathological parameters were analysed in the selected 70 cases and immunohistochemical expression of maspin was studied in different clinicopathological features of the cases includedand it was compared with different studies.

Of the 70 cases, 10 were of normal colonic mucosa,10 cases were ulcerative colitis, 10 cases were non specific colitis and remaining 40 cases were adenocarcinomas which included 4 cases of adenoma with malignant transformation.

Maspin is a 42 kDa protein which belongs to the family of serine peptidase inhibitor proteins. Early experiments with maspin in mammary tissue indicated that it acted as a tumor suppressor. However, maspin does not undergo the S (stressed) to R (relaxed) conformational transition which characterizes active serpins. Thus, the mechanism with which it exerts its tumor suppressor activity has been actively sought.

One of the mechanisms which was proposed to explain the tumor-suppressive functions of maspin in noncolon-derived cells is that it sensitizes cells to apoptosis, thus preventing the clonal expansion of preneoplastic cells with DNA damage. In contrast to the mammary tissue, maspin expression in colon epithelial tissue appears to be related to increased colon cancer risk and decreased patient survival. High maspin expression is associated with neoplastic transformation and high tumor grade .

In our study increased maspin expression was associated with male sex, increasing depth of invasion and presence of lymphatic and vascular invasion thus showing increased expression with increase in aggressiveness of colorectal cancers. No significance was found to be associated with increasing tumor size or differentiation of the tumor or presence of liver metastasis. Our study is comparable with other parallel studies where expression of maspin was studied in colorectal carcinoma and significant co relation with tumor progression and aggressiveness was found .Thus maspin expression was correlated with poor prognosis in colorectal carcinomas and a significant difference in expression was found from normal colonic mucosa to chronic active IBD to carcinoma.

Table 35

Summary table of the findings in the study

Study parameter – maspin expression with respect to -	**P value**
Age	Not significant
Sex	**Significant**
Site	Not significant
Normal colonic mucosa to ulcerative colitis to carcinoma	**Significant**
Tumor size	Not significant
Depth of invasion	**Significant**
Differentiation	Not significant
Lymphovascular invasion	**Significant**
Liver metastasis	Not significant
Lymph node metastasis	Not significant
Survival rates	**Significant**

All these findings were compared with appropriate studies and conclusions were drawn.

SUMMARY AND CONCLUSION

70 cases of colorectal diseases were selected for the study from the period 2012-2013. Among these cases

2.10 cases belonged to non specific colitis ,

2.10 cases to ulcerative colitis

3. 40 cases to colorectal carcinomas

4. 10 were of normal colonic mucosa which served as controls.

Immunohistochemical expression of maspin was studied in all these cases. Maspin, a serine protease inhibitorhas been found to be involved in processes that are important to tumor growth and metastasis such as cell invasion ,apoptosis and angiogenesis.

The expression of maspin with various clinicopathological parameters was analysed and its importance as a prognostic factor was assessed.

Increased Maspin Expression was found to be associated with

1. **Male sex**
2. **Increasing depth of tumor**
3. **Presence of Lymphatic and vascular invasion**
4. **Increased expression in colorectal carcinoma compared to normal colonic mucosa, non specific colitis and ulcerative colitis**
4. **Poor survival**

All these findings were comparable with several studies.

The expression of maspin was not significant in the following parameters

1. Tumor differentiation
2. Liver metastasis
3. Tumor size.

Thus in our study maspin was found to be associated with increasingaggressiveness of colorectal cancers. In a study by Dietmeir et al maspin expression was found to be associated with better response to 5-Fluorouracil based therapy[60] . Thus its expression can be studied in aggressive tumors to decide the line of treatment.

Hence to conclude, immunohistochemical analysis of maspin can be done in non neoplastic colorectal diseases to assess any disease flare and in colorectal diseases to assess its aggressiveness and decide the line of treatment.

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MASTER CHART

BIOPSY NO	AGE	SEX	SPECIMEN TYPE	Diagnosis - Site	Tumor size	Depth of invasion	Lymphatic (L)vascular(V) invasion	Differentiation	Liver Metastasis	Lymph Node metastasis	Maspin Expression
3863/12	50	M	Biopsy	Adenocarcinoma-Cecum	< 4 cms	Above submucosa	-	Moderate	Absent	Absent	1+
1007/13	50	M	Biopsy	Adenocarcinoma-Rectum	< 4 cms	Above submucosa	-	Well	Absent	Absent	2+
2298/12	48	M	Biopsy	Adenocarcinoma-Rectum	< 4 cms	Above submucosa	-	Moderate	Absent	Absent	1+
2819/12	74	M	Biopsy	Adenocarcinoma-Sigmoid colon	< 4 cms	Above submucosa	-	Moderate	Absent	Absent	1+
3750/12	55	M	Biopsy	Adenocarcinoma - Rectum	< 4 cms	Above submucosa	-	Moderate	Absent	Absent	-
1127/13	66	F	Biopsy	Adenocarcinoma - Rectum	< 4 cms	Above submucosa	-	Well	Absent	Present	1+
1117/13	50	M	Biopsy	Adenocarcinoma -Sigmoid colon	< 4 cms	Above submucosa	-	Well	Absent	Present	1+
4302/12	74	M	Biopsy	Adenocarcinoma-Rectum	< 4 cms	Above submucosa	-	Poorly	Absent	Absent	-
4240/12	72	F	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Below subserosa	-	Moderate	Absent	Absent	3+
3818/12	27	F	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Muscularis	L +	Well	Absent	Absent	1+
503/12	51	M	Hemicolectomy	Adenocarcinoma-Hepatic Flexure	> 4 cms	Below subserosa	L +, V +	Moderate	Present	Absent	-
1696/12	57	F	Proctocolectomy	Adenocarcinoma -Rectum	> 4 cms	Below subserosa	L +, V +	Poorly	Absent	Absent	3+
3404/12	52	M	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Below subserosa	L +	Moderate	Absent	Absent	2+
3785/12	75	M	Hemicolectomy	Adenocarcinoma-Cecum	> 4 cms	Muscularis	-	Well	Absent	Present	1+
3547/12	72	F	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Below subserosa	-	Moderate	Absent	Absent	2+
3991/12	50	M	Hemicolectomy	Adenocarcinoma-Sigmoid colon	> 4 cms	Muscularis	-	Moderate	Absent	Present	-
1303/12	60	M	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Below subserosa	L +	Moderate	Absent	Absent	2+
892/12	55	M	Hemicolectomy	Adenocarcinoma-Cecum	> 4 cms	Muscularis	-	Poorly	Absent	Absent	1+
2863/12	70	M	Hemicolectomy	Adenocarinoma-Hepatic flexure	> 4 cms	Below subserosa	L +, V +	Moderate	Absent	Absent	3+
4355/12	65	M	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Muscularis	-	Moderate	Absent	Present	1+
5969/12	60	M	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Below subserosa	L +	Moderate	Absent	Absent	1+
2177/12	57	M	Hemicolectomy	Adenocarcinoma-Hepatic Flexure	> 4 cms	Muscularis	L +	Moderate	Absent	Present	1+
5861/12	55	M	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Muscularis	-	Moderate	Absent	Absent	1+
3077/12	20	M	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Below subserosa	-	Moderate	Absent	Present	1+
1573/12	42	M	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Below subserosa	L +, V +	Moderate	Present	Absent	3+
4293/12	41	M	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Below subserosa	L +, V +	Moderate	Absent	Absent	3+
4175/12	65	M	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Below subserosa	-	Poorly	Absent	Absent	1+
3195/12	48	F	Hemicolectomy	Adenocarcinoma-Ascending colon	> 4 cms	Muscularis	L +	Moderate	Absent	Absent	2+
2378/12	57	M	Hemicolectomy	Adenocarcinoma-Ascending colon	> 4 cms	Below subserosa	-	Moderate	Absent	Present	2+
3894/12	65	M	Hemicolectomy	Adenocarcinoma-Descending colon	> 4 cms	Below subserosa	-	Moderate	Absent	Present	2+
4388/12	60	F	Hemicolectomy	Adenocarcinoma-Ascending colon	> 4 cms	Below subserosa	-	Moderate	Absent	Absent	3+
589/12	43	M	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Below subserosa	-	Moderate	Absent	Absent	3+
618/12	65	M	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Muscularis	-	Well	Absent	Absent	2+
1367/12	70	M	Proctocolectomy	Adenocarcinoma-Rectum	> 4 cms	Muscularis	-	Well	Absent	Absent	2+
1498/12	42	M	Hemicolectomy	Adenocarcinoma-Ascending colon	> 4 cms	Muscularis	-	Well	Absent	Absent	1+
3580/12	50	M	Biopsy	Adenoma-Sigmoid colon	< 4 cms	Below subserosa	-	Moderate	Absent	Absent	2+
3547/12	72	F	Biopsy	Adenoma-Rectum	< 4 cms	Below subserosa	-	Moderate	Absent	Absent	3+
3193/12	56	M	Total Proctocolectomy	Adenoma-Rectum	> 4 cms	Below subserosa	-	Poorly	Absent	Absent	2+
4102/12	72	F	Proctocolectomy	Adenoma-Rectum	< 4 cms	Muscularis	-	Well	Absent	Absent	2+
749/12	40	M	Biopsy	Non Specific colitis-Descending colo	-	-	-	-	-	-	-
809/12	47	M	Biopsy	Non Specific colitis-hepatic flexure	-	-	-	-	-	-	-
847/12	47	M	Biopsy	Non Specific colitis-hepatic flexure	-	-	-	-	-	-	-
690/12	26	M	Biopsy	Non Specifc colitis-Cecum	-	-	-	-	-	-	-
506/12	30	M	Biopsy	Non Specific colitis-hepatic flexure	-	-	-	-	-	-	1+
2296/12	30	M	Biopsy	Non Specific colitis-Rectum	-	-	-	-	-	-	-
749/12	40	M	Biopsy	Normal colonic mucosa	-	-	-	-	-	-	-
809/12	47	M	Biopsy	Normal colonic mucosa	-	-	-	-	-	-	-
847/12	47	M	Biopsy	Normal colonic mucosa	-	-	-	-	-	-	-
690/12	26	M	Biopsy	Normal colonic mucosa	-	-	-	-	-	-	-
506/12	30	M	Biopsy	Normal colonic mucosa	-	-	-	-	-	-	1+
2296/12	30	M	Biopsy	Normal colonic mucosa	-	-	-	-	-	-	-
3186/12	51	M	Biopsy	Ulcerative colitis-Rectum	-	-	-	-	-	-	-
4281/12	37	F	Biopsy	Ulcerative colitis-Cecum	-	-	-	-	-	-	2+
759/12	45	F	Biopsy	Ulcerative colitis-Rectum	-	-	-	-	-	-	2+
1531/12	18	M	Biopsy	Ulcerative colitis-Rectum	-	-	-	-	-	-	-
3059/12	26	M	Biopsy	Ulcerative colitis-Cecum	-	-	-	-	-	-	1+
964/12	30	M	Biopsy	Ulcerative colitis-Cecum	-	-	-	-	-	-	-
3186/12	51	M	Biopsy	Ulcerative colitis-Rectum	-	-	-	-	-	-	-
4281/12	37	F	Biopsy	Ulcerative colitis-Rectum	-	-	-	-	-	-	2+
3059/12	26	M	Biopsy	Ulcerative colitis-Transverse colon	-	-	-	-	-	-	1+
3059/12	26	M	Biopsy	Non Specific colitis-Rectum	-	-	-	-	-	-	1+
1499/12	39	M	Biopsy	Non Specific colitis-Cecum	-	-	-	-	-	-	1+
1572/12	42	F	Biopsy	Non Specific colitis-Cecum	-	-	-	-	-	-	-
4202/12	25	M	Biopsy	Non Specific colitis-Sigmoid	-	-	-	-	-	-	-
2690/12	26	M	Biopsy	Non Specific colitis-Ascending colon	-	-	-	-	-	-	-
1499/12	39	M	Biopsy	Normal colonic mucosa	-	-	-	-	-	-	1+

1572/12	42 F	Biopsy	Normal colonic mucosa	-	-	-	-	-	-	-
2690/12	26 M	Biopsy	Normal colonic mucosa	-	-	-	-	-	-	-
4202/12	25 M	Biopsy	Normal colonic mucosa	-	-	-	-	-	-	-

தகவல் படிவம்

தங்களுக்கு செய்த பெருங்குடல் அராய்ச்சி மூலம் தங்களுக்கு பிரச்சனை உள்ளது கண்டுபிடிக்கப்பட்டுள்ளது. அதற்கான காரணம் அறிய ஆய்வு மேற்கோள்ளப்பட உள்ளது. இதில் தங்களது நோய் குறித்து விவரங்கள் இதர சதை ஆராய்ச்சி முடிவுகளை தங்கள் சம்மதத்துடன் இவ்வாய்வில் பயன்படுத்த விரும்புகிறோம். பின்னாளில் மீண்டும் ஆய்வில் பங்கேர்க்கவும் தங்கள் சதை இவ்வாய்வில் தங்கள் முழு சம்மதம் பெற்ற பின்னர் மட்றும் மேற்கோள்ளப்படும்.

தங்கள் விரும்பினால் இவ்வாய்வில் இருந்து எப்பொழுதது வேண்டுமானாலும் எந்த சாட்சிகளுக்கும் உட்படலம், விலகிக்கொள்ளலாம்.

இவ்வாய்வில் மூலம் கிடைக்கும் தகவல்களும், பரிசோதனை முடிவுகளும் தங்களின் ஒப்புதல் மூலம் மட்டுமே ஆய்வில் பயன்படுத்த படும்.

ஆய்வாளரின் கைஒப்பம் :

இடம் :

நாள் :

ஆய்வளரின் பெயர் :

சுய ஒப்புதல் படிவம்

பெருங்குடல் பற்றி ஒரு ஆய்வு

ஆராய்ச்சி நிலயம் : நோய்க் குறியியல்த் துறை

ஸ்ட்டான்லீ மருத்துவ கல்லூரி ,

சென்னை- 600001

பங்கு பெருபவரின் பெயர்:

பங்கு பெருபவரின் எண் :

மருத்துவம் ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டுள்ளது. எனது பெருங்குடல் சதை ஆய்வு பற்றிய சந்தேகங்களை கேட்கவும் அதற்கான தகுந்த விளக்கங்களை பெறவும் வாய்ப்பளிக்க பட்டது.

நான் எனது சதையை இவ்வாய்வில் பயன்படுத்த தான் இச்சையாக சம்மதிக்கிறேன். எக்காரணத்தாலும் எந்த கட்டத்திலும் எந்த சாட்சிகளுக்கும் உட்படாமல் நான் இவாய்வில் விலகிக்கொள்ளலாம் என்றும் அறிந்து கொண்டேன்.

இந்த ஆய்வு மூலம் கிடைக்கும் தகவல்களையும் பரிசோதனை முடிவுகளையும் மருத்துவர் மேற்கொள்ளும் ஆய்வில் பயன் படுத்திகொள்ளவும் அதை பிறசுரிக்க்வும் நான் முழு மனதுடன் சம்மதிக்கிறேன். எனக்கு கொடுக்கப்பட்ட அறிவுரைகளின் படி நடந்து கொள்வதுடன் இந்த ஆய்வு மேற்கொள்ளும் மருத்துவருக்கு உண்மையுடன் இருப்பேன் என்றும் உறுதி அளிக்கிறேன்.

பங்கு பெறுபவரின் கைஒப்பம் : **இடம் :**

நாள் :

கட்டை விரல் ஒப்பம் :

பங்கு பெறுபவரின் பெயர் மட்டும் விலாசம் :

INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work : Immuno histo chemical analysis of Maspin expression in chronic Non Neo plastic and Neoplastic colorectal diseases.

Principal Investigator : Dr. R Deepa

Designation : PG in MD (Pathology)

Department : Department of Pathology
Government Stanley Medical College,
Chennai-01

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 08.11.2013 at the Council Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

K Vasanthan

MEMBER SECRETARY,
IEC, SMC, CHENNAI

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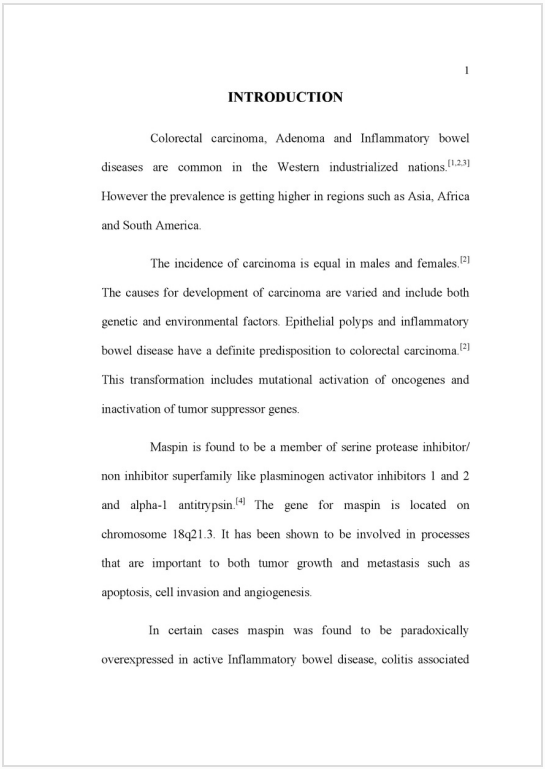

1

INTRODUCTION

Colorectal carcinoma, Adenoma and Inflammatory bowel diseases are common in the Western industrialized nations.[1,2,3] However the prevalence is getting higher in regions such as Asia, Africa and South America.

The incidence of carcinoma is equal in males and females.[2] The causes for development of carcinoma are varied and include both genetic and environmental factors. Epithelial polyps and inflammatory bowel disease have a definite predisposition to colorectal carcinoma.[2] This transformation includes mutational activation of oncogenes and inactivation of tumor suppressor genes.

Maspin is found to be a member of serine protease inhibitor/ non inhibitor superfamily like plasminogen activator inhibitors 1 and 2 and alpha-1 antitrypsin.[4] The gene for maspin is located on chromosome 18q21.3. It has been shown to be involved in processes that are important to both tumor growth and metastasis such as apoptosis, cell invasion and angiogenesis.

In certain cases maspin was found to be paradoxically overexpressed in active Inflammatory bowel disease, colitis associated

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